The Science of Cleaning and Decontamination

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ABSTRACT

Healthcare associated infections (HCAIs) are a growing issue and represent a leading cause of morbidity and mortality. Any patient within the clinical space is at risk, though patients undergoing invasive procedures and admitted to intensive treatment units are at an even greater risk of acquiring HCAIs. An increase in antimicrobial resistance means some HCAIs are becoming more difficult to treat, therefore it is critical any source of HCAI is identified and addressed, not limited to hand hygiene alone. Historically, surfaces and the environment were deemed to play a negligible role in the transmission of HCAIs. It is now clear that surfaces do play an important role, and good environmental cleaning and hand hygiene are critical to prevent this surface-mediated transmission.

We know that hand hygiene saves lives. This has been a topic of debate since the mid-1800s and there are large-scale interventions, audit re-audit studies to improve hand hygiene, with large supportive governmental funding. With environmental surfaces, however, conflicting evidence and determination of wider surfaces being non-critical has left surfaces as a forgotten entity. While, at the same time, HCAI is on the rise. The end goal of this thesis is to provide healthcare professionals with the tools needed to clean and assess their surfaces effectively to reduce HCAI. In order to do so, through a number of experiments and literature assessments, this thesis sought to address the issue of knowing how to sample surfaces, with all the different and sometimes contradictory literature on surface sampling devices, often in an inaccessible format. To address this gap, as well as sampling device testing to contribute to the literature, a literature review of surface

sampling devices published as a guide, open access and freely available, with a how-to sampling poster as a single reference resource.

Surface cleaning is vital to preventing the transmission of these infections, though currently within the UK the requirement is to fulfil a 'visibly clean' standard only, which does not reflect microbiological risk. Environmental surface sampling is an excellent tool to assess how well cleaning has been undertaken, or to identify specific risk by individual pathogen detection. However, routine environmental monitoring is not mandated for clinical environments, and knowing when, how, and what to sample with is lacking evidence. As hospitals and their surfaces are a busy, dynamic environment, knowing how to sample these surfaces can be difficult and confusing.

Studies show there are cleaning failures. However, hospital wards are dynamic environments and patient care does not stop for cleaning. Multiple factors within a ward can skew results. Busy wards are difficult to assess as spot cleaning and movement within the ward is continuous, therefore assessing cleaning performance can be difficult. In paediatric wards, this can be even more challenging, as the patient population interacts differently with the wider surface environment. Current literature assesses cleaning while clinical practise is underway. This means increase of microbial loading on a surface cannot be directly attributed to lack of cleaning. This thesis produced the first study assessing cleaning within a paediatric ward by taking samples directly before and after cleaning, due to the nature of the ward closing before cleaning takes place. This allowed a deeper insight into specifically how cleaning failed. This data has been used for the production of an effective

cleaning training intervention, and also helped support and inform training and set centralised standards for cleaning contractors.

This thesis aims to address the important role surfaces play in the transmission of healthcare associated infection, explore how an infectious agent may move and be deposited within a clinical space, and to address how to mitigate this risk through surface sampling and cleaning. The currently available literature for environmental monitoring and cleaning will be used a starting point for establishing guidelines for cleaning training and surface sampling. Links between patient isolates and the environment can be revealed with typing studies. Potential transmission from the surface environment to near-patient objects and cleaning failures can be shown by microbiological sampling. Though neither of these methods can track the entire route, origin, and journey of a potential pathogen or surrogate pathogen around a ward environment and to quantify how much of an organism has been transferred. This data is needed to drive evidence-based cleaning and sampling interventions, and to also inform safe hospital design. To address this need, this thesis built on two previous studies using an oligonucleotide marker by developing a safer marker with no resistance gene. Previous studies did not quantify recovery as the method design only allowed for presence-absence testing of the oligonucleotide and sampled a very small range of environmental surfaces. This thesis used complex method design so recovery could be quantified, interactions between three separate markers could be assessed, and sampled sites across the entire ward.

This thesis sought to evaluate, develop and apply methods to study microbial presence on surfaces and their removal, by establishing the current state of the

literature and evidence, identifying gaps, and adding to the literature where possible with surface sampler efficacy testing, cleaning agent testing, and to use all this information to suggest guidelines for both surface sampling, cleaning, and cleaning training. Study of the movement and presence of pathogens within the clinical environment was also used to inform cleaning training interventions. There is a need for better advocacy for more frequent, comprehensive and targeted cleaning training for healthcare professionals. This thesis added to the literature concerning cleaning training. Much of the literature concerns resource-heavy large scale cleaning training interventions. While these interventions are multi-faceted and powerful, this does not always represent the reality for some hospital environments. When considering the UK, the NHS and the paediatric critical care environment, cleaning training must be practical, direct and effective and cheap. Patients requiring more careful and continuous care, especially in ICU or CICU, mean nurses cannot leave their patients to attend cleaning training events. The targeted, bed-side design and small-scale nature of the training designed based on a preliminary audit undertaken within the study provides not only a template for future cleaning training (published and available open access) but as a unique insight into how targeting areas of weakness with a small intervention can still be effective and yield improvement in cleaning compliance.

IMPACT STATEMENT

The work within this thesis was designed to support and impact professional practice within the clinical environment, to be used by healthcare professionals both inside and outside of infection control, to make safer and more informed decisions on keeping their surface environment clean and safe to reduce the burden of healthcare associated infection.

The immediate impacts from this thesis are local to the hospital the research was undertaken in, at Great Ormond Street Hospital for children. Research methods were designed for this space and delivered within this hospital. Other outputs from the thesis were national and international, with evidence-based data being published for the wider scientific community. While the data may not be explicitly applicable globally (paediatric setting, publicly funded NHS hospital in a developed country) the key messages remain the same.

The outputs from the thesis were via conference presentation, journal articles, training presented formally (as part of audit re-audit) or informally within the hospital environment. This thesis successfully highlighted how important it is for academics, infection control teams and ward staff to cooperate and work together to make the environment safer. A focus of this research was to ensure all outputs were presented back to the ward staff in an accessible format, to make them aware of the impacts of such research and to promote learning and foster a friendly and open relationship between frontline staff and academic teams.

This cumulated in the following individual impacts;

1. This research established the safe use of an oligonucleotide tracking system for assessing the movement of hospital infection around surfaces. This is an improvement on commonly used marker based systems (ATP, UV) as the oligonucleotides can provide both qualitative and quantitative data, as well as providing several markers allowing the individual marker origin to be identified.
2. A cleaning training package was designed and delivered for surface clinical cleaning. This training was validated as effective as a small-scale effort, and can be re-used in other clinical environments for training healthcare staff. The training package comprised of flashcards and posters, of which are freely available for download for all those who wish to use it.
3. Research into surface sampling of the hospital environment not only identified potential reservoirs for infection in paediatric environments, but also examined the large gaps in the literature when deciding which sampling method to use, both in clinical and non-clinical environments.
4. This study identified how effective commercially available hospital cleaning wipes are. The results revealed how some wipes do not work as

advertised, and that their efficacy may be overstated if they are tested to the current standard.

5. The combination of projects within this study all form a comprehensive whole to push for evidence-based cleaning in the clinical environment, by showing how infection moves around the surface environment, where it is deposited, and how much is deposited.
6. The cleaning review provided an overview to demystify clinical cleaning for those who need it the most. This review pulled together an extensive search of the literature and worked it into a user-friendly and informative guide for clinical teams trying to choose the best cleaning agents for their environment.
7. A sampling study within the clinical environment identified how personal perception of cleaning staff and clinical staff have an impact on cleaning efficacy. This can be used to inform future training packages and allow them to be more effective.

THESIS OUTLINE

The Science of Cleaning and Decontamination

Microbiological Surface Sampling

CHAPTER 2
Surface sampling literature review

CHAPTER 3
Surface sampler efficacy testing

Effective Cleaning

CHAPTER 4
Systematic review of surface cleaning agents and wipe testing

CHAPTER 5
Efficacy testing of wipes and liquid cleaning agents

CHAPTER 6
Ward sampling to assess cleaning efficacy and organism spread

Behaviour and Training

CHAPTER 7
Observational study with cleaning training intervention

CHAPTER 8
Validation of oligonucleotide

CHAPTER 9
Oligonuclotide ward release

This thesis consists of three themes; microbiological surface sampling (chapters 2 and 3), effective cleaning (chapters 4, 5 and 6) and behaviour and training (chapters 7, 8 and 9).

Chapter 1 explores the background to surfaces and Healthcare Associated Infections (HCAIs), how pathogens can survive on surfaces, and the options for assessing hospital surfaces. Chapter 2 explores the associated difficulties in sampling these surfaces, by use of a comprehensive literature review. With these gaps in the literature identified, chapter 3 proceeds to test sampling devices under different

conditions with different organisms. As the importance of cleaning is highlighted, chapter 4 is a systematic review of surface cleaning agents, exploring what is best to clean different clinical surfaces. An assessment of commercially available surface wipes and liquid cleaning agents is undertaken in chapter 5. To continue assessing cleaning, and explore how cleaning really happens in the clinical environment, chapter 6 is a ward-wide sampling study to assess cleaning efficacy. Chapter 7 uses best practise obtained from previous chapters to improve cleaning on a ward by use of an observational study and cleaning training intervention. Chapter 8 introduces the concept of the oligonucleotide as a surrogate infection, and shows the various methods to prepare and validate this material for release within the hospital. Chapter 9 is the release of the oligonucleotide within a ward environment, to identify how pathogens may move across clinical surfaces. Chapter 10 provides a summary of the work undertaken within this thesis, the main findings, and highlights limitations and where future work could take place.

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ABBREVIATIONS

ACC	Aerobic colony count
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
ATP	Adenosine tri-phosphate
CaMV	Cauliflower mosaic virus
CDC	Centers for Disease Control
CEGE	Civil, Environmental and Geomatic Engineering
CFU	Colony forming units
CFU/cm^2	Colony forming units per centimeter squared
CFU/ml	Colony forming units per millilitre
CICU	Cardiac intensive care unit
ClO2	Chlorine dioxide
cm	Centimeter
CoN	Coagulase negative staphylococci
DNA	Deoxyribonucleic acid
EU	European Union
FDA	US Food and Drug Administration
GOSH	Great Ormond Street Hospital
GP	General practitioner
HCAIs	Healthcare associated infections
HPV	Hydrogen peroxide vapour
Hrs	Hours
IPC	Infection prevention and control
ISO	International organisation for standardisation
MALDI-TOF	Matrix-assisted laser desorption/ionisation time-of-flight
MDR	Multi-drug resistance

MDRO Multi-drug resistant organism

ml Mililiter

NaClO Sodium hypochlorite

NaDCC Sodium dichlorocyanurate

NCTC National Collection of Type Cultures

NHS National Health Service

^{o}C Degrees Celsius

PCR Polymerase chain reaction

PDR Pan-drug resistance

PPE Personal protective equipment

ppm Parts per million

PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses

qPCR Quantitative polymerase chain reaction

QUAT Quaternary ammonium compound

RLU Relative light units

RPM Rotations per minute

RTU Ready-to-use

TSA Tryptone soya agar

TSB Tryptone soya broth

UCL University College London

UK United Kingdom

µl Microliter

USP United States Pharmacopeia

UV Ultra violet light

WHO World Health Organisation

Chapter 1 INTRODUCTION AND LITERATURE REVIEW

1.1 PROBLEM STATEMENT

Healthcare associated infections (HCAIs) are defined by the NHS as infections that 'develop either as a direct result of healthcare interventions such as medical or surgical treatment, or from being in contact with a healthcare setting' [1]. These infections occur when a pathogen comes into contact with a patient, from either the patients' own microbial flora, classified as endogenous origin, or from the wider environment facilitated by the movement of healthcare workers, classified as exogenous origin [2]. For exogenous acquisition, the role of surfaces in the transmission of HCAIs has been heavily debated, and historically, were deemed to play a negligible role. Surfaces were classed as non-critical, and surfaces within the wider environment, such as walls and floors, are left forgotten while the focus remains on high-touch surfaces closer to a patient. This has held back the understanding of the role surfaces play in HCAI, and has attributed to the lack of evidence-based guidance on microbiological surface sampling for clinical environments, and efficacy testing of surface sampling devices. Surfaces within the wider environment, not near a bedspace or classified as high-touch objects, are often left forgotten. Cleaning is a key intervention in order to control or mediate environmental transmission risk, however, poorly designed or implemented cleaning practices can lead to poor and infrequent cleaning of these surfaces, which can lead to transmission of HCAI across hospital surfaces.

There are multiple options for surface cleaning, and the wide range of clinical settings and surface types require different types and levels of cleaning in order to keep the environment safe [3, 4]. Different surface materials and surface types have an impact on how well cleaning is performed, both by personal perception of a surface and how to approach the cleaning, as well as the inherent ease in which different surface materials can be cleaned. Knowing how to clean, when to clean, and what to clean with can be a challenging undertaking, particularly with the lack of evidence-based guidance for cleaning and cleaning training [3]. This has led to wide variation in what constitutes as cleaning training across different hospitals. Training content, frequency and quality cause variation in cleaning.

Environmental screening can help with identifying the source of infection and show cleaning failures [5, 6]. Environmental screening is based on two goals, either assessing clinical risk or assessing environmental cleanliness. Both goals require different surface sampling approaches, however, the lack of evidence-based guidance can make these assessments difficult.

With a lack of guidance for both cleaning and assessing how well cleaning has been undertaken in the clinical environment, it can be difficult to determine if these surfaces are safe, or if they pose a risk in the transmission of HCAI.

1.2 AIMS AND OBJECTIVES

This study aims to explore the different components within the science of cleaning and decontamination, both in how to clean hospital surfaces, what to clean with, how to assess if surfaces are clean, how different surfaces support effective cleaning, and the roles of behaviour and training related to cleaning. This was achieved with the following objectives;

- To evaluate the options for microbiological surface sampling of the clinical environment by review of the literature (chapter 2) and in-house sampling device efficacy testing (chapter 3).
- To understand what makes effective cleaning by a systematic review of the literature of cleaning options (chapter 4) and to assess the efficacy of several ready-to-use clinical surface wipes (chapter 5).
- To identify how well cleaning has been undertaken, and the impact of surface composition on a ward environment by ward-wide microbiological sampling before and after cleaning (chapter 6).
- To evaluate behaviour and cleaning training, by an observation study of surface cleaning within the clinical environment of nurses and cleaners, and to see how a specially-designed evidence-based cleaning training package has an impact on how well cleaning is undertaken by an audit re-audit study (chapter 7).
- To assess how an infectious agent may move around the clinical surface environment by use of a surrogate material, validating this surrogate

(chapter 8) and releasing and tracking it within a real ward environment (chapter 9).

1.3 HEALTHCARE ASSOCIATED INFECTIONS

Healthcare associated infections (HCAIs) are defined as infections resulting from a hospital stay, and are associated with increased length of stay, increased antimicrobial use, and in some cases, death [7]. An increasing lifespan and reliance on modern medicine can leave patients susceptible to HCAI through many forms of invasive treatment and the devices used during a hospital stay, such as catheters and ventilators [7]. In Europe, HCAIs have been attributed to 37,000 deaths per year, and 25,000 people per year die from hospital-acquired resistant infections [8]. In surgical intensive care units (ICU), mortality from central line-associated bloodstream infections can be as high as 35% [9]. Patients in ICU that are critically ill are more likely to acquire HCAI, with data suggesting that up to 51% of ICU patients acquire HCAI during their stay, of which 71% receive antimicrobials [10]. HCAI incidence more than doubles this ICU mortality rate [10].

The future of HCAI treatment is looking bleak; more multi-drug and even pan-drug resistant organisms are being found in the clinical environment [11, 12], This is exampled by the first concern of nosocomial outbreak of carbapenem-resistant *Acinetobacter baumannii* in a New York hospital in 1991 [13], in which environmental samples (including beds, tables, IV drip holders, patient monitoring equipment and ventilators) were found to be positive with multi-drug resistant *A. baumannii*. While these positive environmental samples could not be directly linked to the reported clinical infection, the hands of the healthcare workers were also found to be positive [13]. There are increasing reports today of such MDRO's being recovered from hospital environmental surfaces, while the burden on current antimicrobials is at an

all-time high. There is growing concern that in the very near future, pan-drug resistant (PDR) infections will increase, as nosocomial outbreak of pan-drug resistant infection has already been identified [11] and that we will be reaching a post-antimicrobial-era [14].

Despite an increased focus on protecting our remaining antibiotics, and an increase in antimicrobial stewardship, the situation is yet to significantly improve. In the past 10 years, a 'discovery void' has occurred, in stark contrast to the significant and continual discovery and development of new antimicrobials between the 40's and 60's [15]. Discoveries have stalled, and improper prescribing, poor use and unlicensed distribution of antimicrobials across the world is worsening this issue as poor stewardship is reducing the power of the remaining antimicrobials, while new discoveries of novel antimicrobials cannot keep up with the demand [16]. Soon, untreatable infections may be commonplace within the hospital, and invasive procedures ever more risky. As such, the risk the clinical surface environment poses to patients and risk of HCAI acquisition is ever increasing.

There are many organisms associated with the ability to cause HCAI. Most notably, are the 'ESKAPE' pathogens, (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) classified for their antibiotic resistance and ability to 'escape' the biocidal action of antibiotics [17]. These pathogens are associated with significantly higher rates of morbidity and mortality and represent the ever-growing issue of HCAI and antimicrobial resistance. The work within this thesis is undertaken within the paediatric environment. Paediatric represent a clinically vulnerable cohort, and are

at greater risk of morbidity and mortality. This population subset also has implication for antimicrobial use and efficacy; some adult antimicrobials are simply not suitable for paediatric patients, as pharmacokinetic and pharmacodynamics vary between adults and children [18]. Because of these factors, advocacy of antimicrobial stewardship in relation to paediatrics specifically is rising [19].

However, organisms that can cause HCAI in a clinical setting can be relatively innocuous. Organisms such as *Staphylococcus epidermidis* and *Candida albicans* are common constituents of the normal human skin and gut flora, however, introduction by catheters and central lines can allow these organisms to cause HCAI, such as urinary tract infections and septicaemia [20, 21].

1.3.1 ***STAPHYLOCOCCUS AUREUS***

Staphylococcus aureus is a well-known leading pathogen that can lead to HCAI [22]. In 2005 and 2011, mandatory reporting for bacteraemia cases caused by methicillin resistant (MRSA) and methicillin sensitive *S. aureus* (MSSA) was introduced for the NHS. In 2018, 805 and 12,073 bacteraemia cases were reported caused by MRSA or MSSA [21, 23]. It is subject to extensive study, and is one of the most commonly researched pathogens in relation to hospital surfaces according to search data from ScienceDirect [23]. *S. aureus* is commonly present in healthy individuals, residing in the nares of approximately 30% of the population [24]. This colonisation can lead to translocation and allow infection. Translocation is the movement of organisms from one site of the body to another, such as the movement or migration of commensal organisms or indigenous gut flora to usually sterile tissues and internal organs, facilitated by many factors not limited to stress, host immune

deficiencies, immunosuppression or disturbance of gut flora [25, 26]. *S. aureus* can survive and persist well on clinical surfaces. With the increase of MRSA in hospitals, measures were introduced to try to reduce the prevalence of MRSA within the clinical environment, including screening and isolation of shedding patients. Shedding is most common in infected patients [27] These measures have been largely successful, and has led to an overall reduction of hospital-acquired MRSA [28]. By the 2020-2021 reporting cycle, reports of MRSA and MSSA had reduced by 15% and 3.4% respectively since the 2018 report [21]. Shedding is the excretion or release of an organism from a patient such as from saliva, skin cells or faeces.

1.3.2 ***KLEBSIELLA PNEUMONIAE***

Klebsiella pneumoniae is a pathogen of clinical significance that survives well in the clinical environment, favouring wet environments such as sink areas and surfaces within bathrooms. Recent studies have demonstrated that hospital sinks can be contaminated with carbapenemase-producing *K. pneumoniae [29-31]*. Between 2018-2019, 21,275 *Klebsiella* spp. bacteraemia cases were reported within the NHS [32]. *K. pneumoniae* account for the majority of *Klebsiella* spp. recovered from patient samples. *K. pneumoniae* readily exchange resistance genes via plasmids between similar species, such as from the Enterobacteriales family [33]. Persistence of β-lactamase resistant *K. pneumoniae* in clinical environments is a growing issue, and these resistant infections are becoming increasingly difficult to treat [33]. *K. pneumoniae* can not only spread easily within the environment, but is associated with resistance to the highest-priority antimicrobial agents [34]. As effective antimicrobials dwindle, resistant organisms such as *K. pneumoniae* are on the WHO

global priority list of antibiotic-resistant bacteria. *K. pneumoniae* was within the priority 1 group [35].

1.3.3 *Enterococcus faecalis*

Enterococcus faecalis, like *S. aureus*, can be found in healthy individuals. Nosocomial infection with *E. faecalis* is associated with severe clinical presentation, such as endocarditis and sepsis [36]. *E. faecalis* becomes a clinical issue when this known commensal organism is translocated, or transferred, to a susceptible area of the body, and introduced through broken skin, or via a medical procedure such as catheterisation [25]. *E. faecalis* is responsible for up to 90% of all enterococcal infections [10]. Voluntary laboratory surveillance of *Enterococcus* spp. in England, Wales and Ireland found *E. faecalis* to be the most commonly reported species (45%) in 2018. In England, 7,347 *Enterococcus* spp. bacteraemia cases were reported, of which 3,249 cases were identified as *E. faecalis* [37].

Additionally, *E. faecalis* is becoming more resistant to different antimicrobial agents [38]. Resistance to vancomycin is on the rise, and many pathogenicity factors have been identified for *E. faecalis* [38], such as the presence of two proteases and cytolysin which promotes appearance in the bloodstream and enhances toxicity to mammalian tissue [39]. This cytolysin operon is located within an *E. faecalis* pathogenicity island and consists of six genes (cylLL, cylLS, cylM, cylB, cylA, and cyl) each of which playing a role in toxin synthesis, modification, secretion, activation, and immunity [39].

1.3.4 ***Pseudomonas aeruginosa***

Pseudomonas aeruginosa is a pathogen of clinical concern that is ubiquitous in the environment. *P. aeruginosa* can persist well on surfaces, and is known for its ability to produce robust biofilms, resisting both the environment and cleaning agents [40]. Outbreaks of *P. aeruginosa* have been linked to water sources within the hospital, such as from bathrooms and hand wash sinks [41-43]. An example of which is an outbreak within a neonatal ICU in 2016 leading to two deaths [42]. Interventions with hyperchlorination of the water and point-of-use filters installed into the taps did not eliminate the transmission. It was determined the source was contaminated plumbing and while not all patient isolates could be directly linked to the plumbing, exposure to the water in the NICU was deemed the most likely cause [42]. A more recent study by Catho *et al.* (2021) used whole genome sequencing to assess the link between beta-lactamase producing *P. aeruginosa* outbreak within an adult ICU [44]. *P. aeruginosa* was isolated from the sink taps and drains and the epidemiological link confirmed between the patient isolates and the sink isolates [44]. This ability to assess the sequence of an organism allows more complete insight into the potential links between specific clinical infection and environmental reservoirs, which is a great advantage of molecular methods (see section 1.4.1).

P. aeruginosa can lead to severe infections, particularly in immunocompromised patients, such as those with cystic fibrosis or open wounds [40] and are associated with significant morbidity and mortality, of up to 38% [45]. *P. aeruginosa* infections in the clinical environment are a real issue. Between 2018-

2019, across 147 NHS trusts, over 4,000 *P. aeruginosa* bacteraemias were reported [46].

1.3.5 Gram-Positive and Gram-Negative Organisms

Bacterial organisms can be broken down into two distinct categories; Gram-positive and Gram-negative. These categories relate to the result following a Gram stain test, which is a method of identifying an organism by colour depending on the cell wall properties. This procedure was pioneered by Hans Gram in 1853 [47]. A staining procedure using crystal violet as a stain, or safranin as a counterstain, reveals if a bacteria is Gram-positive or Gram-negative. A Gram-positive organism is stained by the crystal violet and takes upon a purple colour, due to the presence of the thick peptidoglycan layer [48]. Gram-negative organisms do not have this thicker peptidoglycan, and the alcohol wash step within the staining procedure removes this stain and, instead, the organism picks up the pink counterstain [48]. This is a fast and cheap method of identifying organisms via microscopy analysis including Gram stain, colony size, shape and morphology.

Gram-positive and Gram-negative organisms have different properties, which have implications for pathogenicity, patient risk, cleaning and antimicrobial resistance risk. Gram-negatives, due to the thin cell wall, have increased susceptibility to some cleaning agents. Gram-positive organisms have increased resistance, due to the thick, multilayered cell wall. Gram-positive organisms, such as *Clostridioides,* use sporulation to protect its DNA during times of environmental stress [49]. The cortex, a cross-linked peptidoglycan structure is implicated in the heat resistance from the spore. This allows long-term survival during stress, such as

environmental stressors and exposure to cleaning agents [49]. It is also this thickened cell wall that make Gram-negatives more resistant to antibiotics than Gram-positives [48, 50, 51].

1.3.6 MULTI-DRUG RESISTANCE

Multi-drug resistance (MDR) is defined as an organism demonstrating resistance to at least one antimicrobial agent within three or more categories. Antimicrobial agents are defined as substances, natural or synthetic, that kill or inhibit the growth of microorganisms, including bacteria, fungi and algae [52]. The word 'antibiotic' means a natural product that is toxic to cells, and the term is applied to any drug used to treat microbial infection [53]. Under the umbrella of antimicrobial agents, the following categories are formed; antibiotics, antivirals, antiparasitics and antifungals. Antibiotics can be further broken down into the following categories by mode of action; inhibition of cell wall synthesis (β-lactams including penicillins, cephalosporins, monobactams, glycopeptides such as vancomycin) inhibition of bacterial protein synthesis (aminoglycosides, chloramphenicol, macrolides, tetracycline, streptogramins, linezolid) inhibition of nucleic acid synthesis (flluoroquinolones, rifampin) and inhibition of folic acid synthesis (sulfaonamides, trimethoprim, pyrimethamine)

In 2011, the term pan-drug resistance was coined, meaning an organism is not susceptible to any antimicrobial agent, regardless of the antimicrobial category. With MDRO's on the rise and discovery of new antibiotic antimicrobial agents slowing, this issue is becoming a public health emergency. Antibiotic misuse and poor stewardship has led to MDRO's increasing. Even in the 40's, Fleming, a microbiologist

that first discovered penicillin, was concerned about the misuse of the latest ‘wonder drug’ penicillin leading to reduced efficacy [54]. As a result, by the 50’s this resistance led to such a substantial clinical problem that the new, beta-lactams were discovered and developed [54, 55]. Efforts of antimicrobial stewardship have attempted to slow and combat this growing resistance, yet there are many challenges with attaining good stewardship. A current example of which, despite all our knowledge and concern over the need for strong stewardship, is reflected within the current pandemic, which has many scientists concerned that inappropriate treatment of COVID-19 and over-prescription of antibiotics may exacerbate this issue [56, 57]. The ‘golden age’ of antibiotic discovery ended abruptly in the early 1960’s as discoveries of new agents stalled, and the knowledge of antibiotic action and resistance began to accumulate [16]. The World Health Organisation (WHO) released a list of priority pathogens, including resistant Enterobacteriales, *P. aeruginosa* and *Acinetobacter baumannii* [16]. Isolates of *A. baumannii* resistant to all known antimicrobials have been found [58]. With this increase in MDRO and pan-drug resistance, the importance of preventing these infections is increasing. Surface cleaning and hand hygiene may soon be the only defence against some nosocomial pathogens.

It must then be considered how organisms become resistant. The first important distinction is resistance and persistence. Persistent organisms do not contain the required resistant genes when exposed to an antimicrobial agent. Some cells survive, due to dormancy or biofilm, and are called ‘persister’ cells, or persisters, named by Joseph Bigger in 1944 to ensure they are not confused as resistant cells [59]. Persister cells can account for up to 1% of cells within biofilm and therefore

represent a unique challenge in relation to surfaces; if surfaces are not cleaned frequently enough, persister cells can remain on surfaces and re-grow and lead to nosocomial infection [60-62]. Resistance can be either naturally occurring within an organism and always expressed within a species and not related to horizontal gene transfer, such as natural absence of a target site [63], or induced natural resistance, following exposure to a specific stressor, such as exposure to an antimicrobial agent. These are intrinsic resistance mechanisms, and include reduced permeability of the outer membrane and activity to efflux pumps (table 1.1) [64, 65]. Table 1.1 demonstrates the key ways organisms become resistant to antibiotics.

Table 1.1 Resistance strategies used by organisms taken from CDC 2021 [66]

Resistance Mechanism	Description
Restrict access of the antibiotic	Organisms restrict access by changing the entryways or limiting the number of entryways. **Example:** Gram-negative bacteria have an outer layer (membrane) that protects them from their environment. These bacteria can use this membrane to selectively keep antibiotic drugs from entering.
Get rid of the antibiotic	Organisms get rid of antibiotics using pumps in their cell walls to remove antibiotic drugs that enter the cell. **Example:** Some *Pseudomonas aeruginosa* bacteria can produce pumps to get rid of several different important antibiotic drugs, including fluoroquinolones, beta-lactams, chloramphenicol, and trimethoprim.
Change or destroy the antibiotic	Organisms change or destroy the antibiotics with enzymes, proteins that break down the drug. **Example:** *Klebsiella pneumoniae* bacteria produce enzymes called carbapenemases, which break down carbapenem drugs and most other beta-lactam drugs.
Change the targets for the antibiotic	Many antibiotic drugs are designed to single out and destroy specific parts (or targets) of a bacterium. Organisms change the antibiotic's target so the drug can no longer fit and do its job. **Example:** *Escherichia coli* bacteria with the *mcr*-1 gene can add a compound to the outside of the cell wall so that the drug colistin cannot latch onto it.
Bypass the effects of the antibiotic	Organisms develop new cell processes that avoid using the antibiotic's target. **Example:** Some *Staphylococcus aureus* bacteria can bypass the drug effects of trimethoprim,

Acquired resistance can occur by mutation, or by horizontal gene transfer. Mutations can be spontaneous, which lead to resistance. An example of this would be upregulation of the production of enzymes that inactivate an antimicrobial agent, such as ribosomal methylate in staphylococci [67]. Further example of this would be *S. aureus* producing penicillinase enzyme to destroy penicillin, which is encoded by either chromosomal genes or located on plasmids [68]. Mutations can also be

adaptive, whereby the mutation is not spontaneous or random, but a specific reaction to the environment to improve the chances of survival (table 1.1). An example of this adaptive mutation is the upregulation of the MexAB-OprM efflux pump, which is one of the largest MDR pumps with high-level expression, in *P. aeruginosa* biofilms exposed to membrane-targeting antimicrobials (table 1.1) [69, 70]. Horizontal gene transfer is the primary route of spreading resistance, whereby bacteria 'swap' and exchange genes, transferred by a number of mechanisms, including conjugation, transduction and transformation and incorporating this new material into the host genome or plasmid [63, 67]. Plasmids are double-stranded DNA molecules, usually circular, that are able to replicate independently [71]. They are physically independent of the major bacterial chromosome [71]. Under the process of conjugation, plasmids are transferred via sex pili, leaving both organisms with a copy of the plasmid [72]. This is the most frequent method of conjugation within Gram-negative organisms, and occurs less frequently in gram-positive organisms. In Gram-positive organisms, the most common route is conjugation via transposons [71, 72]. Transposons are an important mechanism as all known conjugative transposons carry a tetracycline resistance gene [71]. Transposons are a transposable genetic element that can move from one DNA sequence to other sites within bacterial cells, and can carry antibiotic genes even without conjugative ability.

These resistance mechanisms need to be considered in relation to the hospital surface environment, and what this means for HCAI. Horizontal transfer can occur between the same species, or between different species or genrea. This means close presence of multiple bacterial communities on a surface can compound the

issue of this gene transfer, particularly as environmental organisms are shown to have large accessory genomes that can act as genetic sinks for antibiotic resistance. While not all of the organisms present may be pathogenic, easily transmissible, such as very high infectious dose needed for clinical infection, or survive well long term on surfaces, sharing of genetic material between these organisms can allow organisms to collect resistance genes and incorporate them into their genome. This abundance of genetic material and distribution of resistance genes within this environmental resistome will increase the population of MDROs within the clinical environment [73]. To counteract this sharing of resistance genes, these organisms should be removed from the surfaces to prevent the exchange of such resistant genes between the same or different species. The solution to this is effective and frequent cleaning (effective cleaning discussed in chapter 4).

1.3.7 ANTIBIOTIC MECHANISMS

Antibiotics work in different ways to treat bacterial infections. They can work broadly, across several types of bacterial species, which are classified as 'broad-spectrum' or they can be more specific and targeted against specific organisms. Broad-spectrum antibiotics are often given when the individual cause of infection is unknown. Simply speaking, antibiotics work by destroying the bacteria, or by preventing the bacteria from reproducing.

β-lactams, which include penicillin, cephalosporins, monobactams and carbapenems are so named as they have a β-lactam ring [68]. The mode of action is bactericidal, by preventing the cell wall production (stopping peptidoglycan synthesis) of gram-positives [74]. Some organisms, such as *E. coli* and *Klebsiella spp.*

are resistant, as they can produce β-lactamase enzyme which destroys the β-lactam ring in the antibiotic, rendering the mode of action useless [75]. This production of the β-lactamase enzyme is the main resistance mechanism to β-lactams, and is well documented in the clinically significant pathogen, MRSA, in which expression of the PBP2a transpeptide is encoded by the *mecA* gene on the staphylococcal cassette chromosome (*SCCmecA*) [76]. This SCC is a mobile genetic element and carries the *mecA* gene coding for methicillin resistance, and is spread by horizontal gene transfer [77]. It is hypothesised that *S. aureus* acquired the *SSCmecA* from coagulase negative staphylococci [78].

Glycopeptides include vancomycin, which is currently the most clinically important glycopeptide antibiotic [79]. Glycopeptides, like β-lactams, inhibit cell wall synthesis [79, 80]. Glycopeptides bind to the d-Ala-dAla C-terminus of peptidoglycan precursors preventing further growth of the peptidoglycan chain and subsequent transglycosylation and transpeptidation steps of cell wall synthesis [80]. In enterococci, the *van* genes encode the resistance phenotype. The transferability of *vanA* and *vanB* genes is the basis for infection control measures to monitor and prevent the spread of vancomycin-resistant enterococci [80].

Quinolones are bactericidal against most Gram-negatives. They work by inhibiting DNA synthesis, which causes rapid cell death [81]. The main targets are DNA gyrase and topoisomerase IV, which are important in the role of DNA structure. Organisms can become resistant to quinolones via efflux pumps; upregulation of genes involved in the removal of toxic agents [82]. *P. aeruginosa* has at least three known efflux pumps associated with fluoroquinolone removal [82].

Macrolide antibiotics include the commonly-used erythromycin, clarithromycin and azithromycin. They are used to treat respiratory tract infections, skin infection and gastrointestinal infections [83] and are the second most commonly prescribed antibiotic in the NHS [84]. Macrolides work by inhibiting protein synthesis by binding to the nascent peptide exit tunnel and occluding it, on the bacterial ribosome [85]. The most common resistance mechanism to macrolides occur by post-transcription methylation of the 23S bacterial ribosomal RNA. This can be via plasmid-mediated or chromosomal resistance [86]. Resistance via chromosomal mutation is not common, as in most bacteria, there are multiple operons that can be targeted. In organisms with low rRNA (rrn) copy numbers, chromosomal mutations have occurred allowing resistance, such as *Helicobacter pylori [84].*

1.4 EXPLORING SURFACES

Surfaces were not always considered an important part of infection prevention and control. Historically, surfaces were deemed to have no role in HCAI transmission. In 1968, Spaulding created his own approach and classification of surfaces, and classified clinical environments and divided them by potential risk [87]. He understood the need and requirement for a benchmark, in order to effectively assess surfaces by risk in relation to cleaning and disinfection requirements [87]. Surfaces were classified as non-critical, such as table surfaces, stethoscopes and other environmental surfaces, as they did not come into contact with broken skin. Semi-critical, such as medical instruments like pelvic exam probes which came into contact with non-intact skin or mucosal membranes. Or, surfaces could be classed as critical, which include medical devices like intraoperative probes or operating clamps

as they came into contact with the bloodstream [87]. Environmental surfaces, which are the focus within this thesis, were classified under Spaulding as non-critical as they do not directly come into contact with the patient or broken skin barriers [87]. This classification of surfaces has long been held in high regard, and is still referred to today, despite some scientists believing the classification is needing an update [88] as surfaces classified as non-critical by Spaulding have been directly implicated facilitating transmission of clinically significant organisms, such as *P. aeruginosa*, leading to death [42]. The Centers for Disease Control (CDC) supported this classification, and in 1987 stated that surface contamination had no role in nosocomial infection [89] due to several studies published in the 1970’s and early 1980’s suggesting there was little to no correlation [90]. Contamination is a broad term that encompasses unwanted materials on a surface, and can be classified into the following categories; microbial contamination, ionic contamination, thin film or molecular contamination (which can be organic or inorganic), or particles [91]. For the purposes of this thesis, unless stated otherwise, contamination is discussed in relation to microbial contamination.

These viewpoints have allowed a continuing disregard for surfaces and their role in HCAI transmission. Now, scientists are beginning to understand the importance of environmental surfaces and HCAI transmission, and are beginning the difficult journey of advocating for enhanced cleaning and environmental monitoring of clinical surfaces. Recent studies have data-driven conclusions that patients can shed nosocomial pathogens onto their surface environment, and that these pathogens can survive and persist in the environment and that transmission to other

patients can occur, facilitated mostly by healthcare workers' hands [90]. However, even now, there is debate on the role of surfaces and infection transmission in the clinical environment, as some scientists argue that additional high-quality studies are needed to clarify the specific role played by surfaces in the spread of nosocomial infection [90] and the historical damage of surfaces once being considered as not contributing to nosocomial infection.

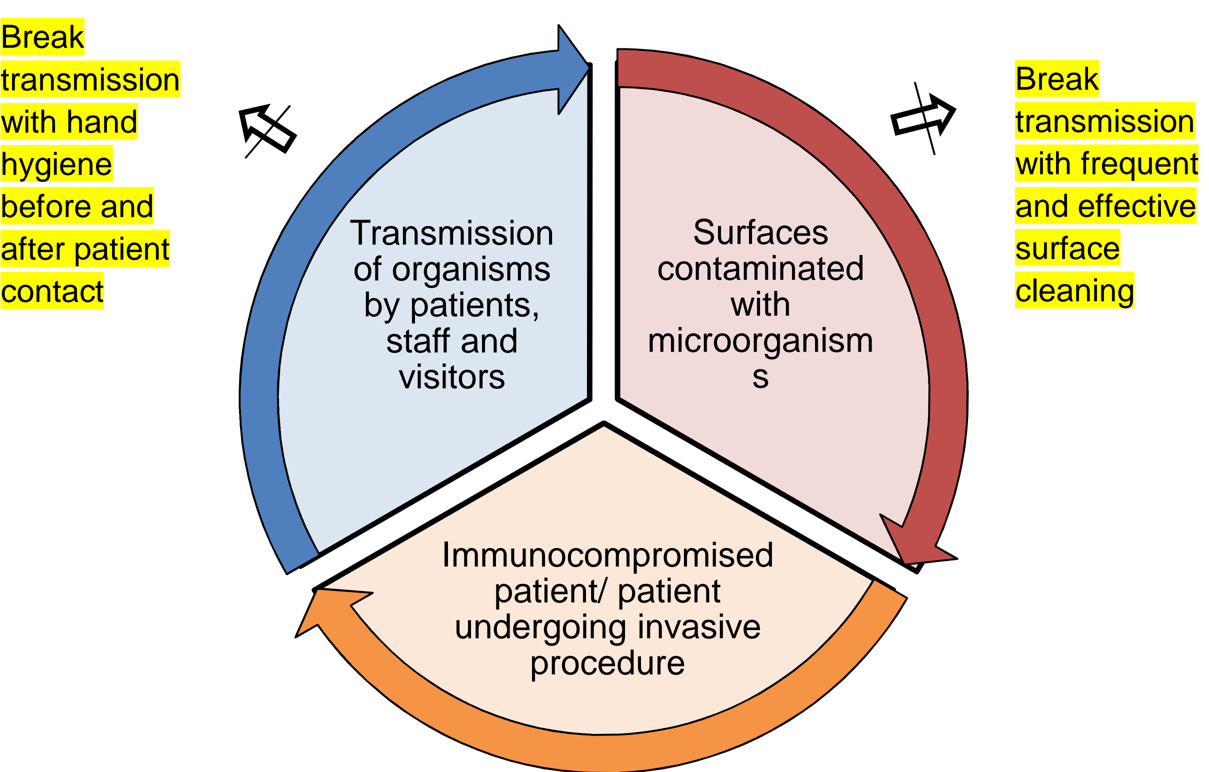


Figure 1.1 Cycle of organism movement around the clinical environment that can lead to HCAI

Studies have shown how microorganisms that can cause infection can move between patients and the environment, facilitated by healthcare workers [92-96]. Figure 1.1 shows the movement of contamination around the clinical environment. Infected or colonised patients shed into their environment, with infected patients representing an increased risk of such shedding [27]. Colonised patients are defined

as patients carrying an organism but without presenting with clinical infection. A common example of this are patients who are colonised with MRSA. Colonisation can, but does not always, lead to infection. Infected patients are patients presenting with clinical infection, where the organism has entered the body. Clinical infection can present as symptomatic or asymptomatic. Both colonised and infected patients can act as reservoirs for infection in the clinical environment [97]. This shedding of organisms into the environment has been considered, along with contaminated healthcare professionals hands, has been attributed to have a major role in environment contamination [27]. An example of the environment acting as a reservoir for contamination is a 20-month retrospective cohort study within a hospital of patient admissions to 8 ICUs, undertaken by Huang, Datta and Platt (2006). The results found that for patients whose prior room occupant were MRSA or VRE positive, the following patients were 1% and 1.7% more likely to acquire MRSA or VRE respectively, when compared with patients where the prior room occupants were MRSA and VRE negative [96]. Another example was a study undertaken in a paediatric intensive care unit in response to an outbreak of imipenem-resistant *A. baumannii*. Extensive environmental monitoring, assessment of clinical samples with isolate typing found the source of the outbreak to be a sink in the PICU [98]. However, not all outbreaks are assessed the same. Without sophisticated molecular analysis, IPC teams rely on a more traditional epidemiological assessment of what happened, looking at when patients began presenting with infection, where the patients had been, and how this might be linked to outbreak. An outbreak of MDR *K. pneumoniae* in an overcapacity (140%) hospital in India found, following environmental screening,

MDR *K. pneumoniae* across samples within the ICU [99]. The most bacterial loading was recovered from humidifiers, refrigerators, incubators, medicine trolleys, trays and boxes, and resuscitation equipment. It was theorised as a most probable cause in relation to microbial loading, that the source was a contaminated fridge where blood bags were stored, and the pathogen was transmitted by healthcare workers hands. It was suggested that this may have been due to improper cleaning of the refrigerator [99]. Without typing studies, however, it is just a probable cause assessment based on the available information IPC teams have. Strong correlations, backed by knowledge of the environment, HCAI incidence data and environmental monitoring can be hypothesised, though correlation does not mean causation. The key for driving change is evidence-based argument, and while making informed correlations between outbreak and individual surfaces is important; this does not provide empirical evidence implicating surfaces with nosocomial infection. Therefore, the gold standard of isolating specific outbreak causes is to use molecular analysis, such as whole genome sequencing (WGS), which allows epidemiological concordance between patient and environmental isolates with high accuracy [100]. This means more reliable conclusions as to the exact surfaces or environments linked to outbreak and patient infection can be made [101]. The cost, logistics and availability of local knowledge of metagenomics to undertake WGS mean not all IPC teams globally can rely on such methods, and revert to more traditional methods of assessing outbreak and their environments [101]. Another factor to consider is the ethical factor relating to genomic data [102]. WGS is most frequently undertaken without patient consent, as seeking consent for all patients involved is not feasible

or practical, though some argue consent is not a concern as there is a moral obligation to act in any way that may reduce HCAI [102, 103]. Surface cleaning, hand hygiene, medical device and clinical equipment sterilisation is required to prevent such infection mediated by surfaces.

The hospital surface environment is host to a multitude of organisms, both pathogenic and non-pathogenic [104-106]. Only a small proportion of these organisms have been identified, and of these, even fewer are culturable. Some environmental organisms simply cannot be cultured in the laboratory environment, classified as viable non-culturable, as they are live but do not grow or divide [107]. Therefore, there are many potential pathogens present on clinical surfaces that can be difficult to determine, unless sampling methods and recovery methods are carefully selected, and the risk of these remains unknown. PCR and qPCR (molecular methods) analysis can give deeper insight into non-culturable organisms, but cannot distinguish between live or dead cells, while agar-based microbiological sampling only recovers live, viable cells which can determine clinical risk, but cannot recover non-culturable organisms. We know that there are organisms persisting on surfaces that we have yet to discover. The first evidence of this was the discovery of microscopy, when it was found that the number of organisms under the microscope far outweighed that which could be grown on the plate [108]. This was called the 'great plate count anomaly' and allowed scientists to understand that not all organisms present could be cultured [109]. In addition, increasingly sophisticated molecular methods began to reveal 16S rNA gene sequences by PCR amplification of DNA sequences from environmental samples [108]. The breadth of diversity within

these samples once again highlighted the lack of characterisation of known environmental species. However, some organisms are well known. Some well-known pathogens associated with HCAIs have been shown to survive and persist in the surface environment.

Surfaces can be assessed in a variety of ways, including with scanning probe microscopy and scanning tunneling microscopy (STM) which allows imaging of the electronic densities of the surface and atomic force microscopy (AFM) which measures the magnitude of attractive or repulsive forces [110]. There is a multitude of surface types, and the hospital environment is host to hundreds of different surface types, made of different materials. The most common materials found within the hospital surface environment are; stainless steel, ceramic tiling, hard plastic surfaces and vinyl flooring. These are the most commonly tested surfaces in relation to assessing clinical cleaning and infection control strategies [111]. These surfaces are so chosen as they are smooth, easy to clean and can withstand harsh cleaning agents without degrading. They fulfil the criteria needed to be hygienic surfaces; smooth, without crevices, folds, pits, and be easy to clean [112, 113]. Rough surfaces promote biofilm and will be harder to clean [113]. The size and shape of certain organisms make it easier for them to form biofilm on surfaces, or to escape cleaning in micro-scratches on a surface (table 3.1).

Stainless steel surfaces are an alloy of pure iron and carbon which make steel, with added chromium, nickel, molybdenum, silicon, aluminium and carbon [114]. Stainless steel makes an excellent material choice for the hospital environment as it requires minimal maintenance, is non-toxic, highly stain resistant and rust resistant.

These properties mean it is easily cleaned [113, 115]. Steel surfaces are classified as having inherent negative surface charge [116], and that thermophilic *Streptococci* cells displaying a negative surface charge were repelled from stainless steel surfaces [117].

Plastics are a range of synthetic or semi-synthetic polymers [118]. Within the clinical environment, plastics are most commonly acrylic or polypropylene [119]. The most commonly used plastics in industry are thermoplastics, named for their ability to be molded and include polypropylene, polyvinyl chloride (PVC) and polyethylene. Plastic surfaces usually carry a negative charge due to the primary dissociation of carboxyl groups formed during polymerisation [120].

Ceramic is a general term use to refer to a range of inorganic materials mostly derived from naturally occurring minerals and hardened in a firing process. Components include clays, kaolins, carbonates, quartz and feldspars. They are usually ionic or covalent bonded materials [121]. Ceramics can refer to porcelain, which has zero apparent porosity and is used within the clinical environment, or earthenware which is used outside of the clinical environment and is typically used for tableware and household goods [121].

Different surface materials have varying properties, which have implications for cleaning and bacterial attachment. Some surfaces are more readily contaminated than other surfaces. It is these surface characteristics, including topography, structure, chemical composition and electronic properties that determine the chemical reactivity of surfaces, which has an impact on the binding ability of organisms in relation to how organisms attach to surfaces (discussed further in

section 3.1.). Certain finishes on surfaces reduce the chemical reactivity of a surface, such as polishing, rendering the surface less susceptible for bacterial attachment from forces such as Van der Waals and electrostatic and hydrophobic interactions [110].

1.4.1 PATHOGENS RECOVERED FROM SURFACES

Many organisms have been recovered from hospital surfaces. Commonly, the most contaminated areas of the hospital are the high-touch surfaces [122, 123] and the majority of the studies find as the surfaces gain vicinity to the patient, contamination will increase [122-124]. Infrequently touched surfaces gave fewer *Clostridioides difficile* positive samples than high-touch surfaces during a ward sampling study [122]. *Clostridioides difficile* is a spore-forming organism known to cause HCAI, and has the ability to persist in the surface environment for long periods of time, estimated up to 5 months [125] and present a challenge for IPC staff as *C. difficile*-positive patients should be isolated to prevent spread [122]. Objects that are located nearest to the patient, such as bed linen, mattresses, pillows, over-bed tables and bed rails are repeatedly reported as highly contaminated [122, 123, 126].

Any movement and interaction involving clinical surfaces represents an opportunity for movement of organisms. There is some evidence that colonised or infected patients shed into their environment; one typing study shows how 70% of the 20 patient and corresponding environmental isolates (n=35) recovered could be linked, either found to be identical or closely related [127, 128]. Sexton *et al.* (2006) Sampled the following surfaces; bed, mattress, linen, table, chair and window ledge, giving a total (N= 502 surface samples) were taken across 4 weeks, of which 53.6%

were found positive for MRSA [127]. Another study by Boyce *et al.* (2015) found that 27% of hospital surface samples (N=350 total samples) were found to be MRSA positive when sampled from rooms of MRSA-positive patients. The following surfaces were sampled; floor, bed linen, patient gown, bed table, blood pressure cuff, bed rail, door handles and the infusion pump button. Environmental contamination of MRSA was found in the rooms of 73% of infected patients and 69% of the rooms from colonised patients [128]. Such typing studies are important when discussing the link between the patient and the surface environment, as this provides deeper evidence that the organisms recovered from the surfaces directly correspond with the patient isolates.

Colonisation refers to an organism living within or on a patient without causing infection or harm. Infection refers to invasion of the host body with an organism, leading to an infection. Colonisation can lead to infection due to translocation of organisms from one site to another, such as the translocation of indigenous gut flora to usually sterile tissues and internal organs, facilitated by many factors not limited to stress, host immune deficiencies, immunosuppression or disturbance of gut flora [25]. Environmental samples taken from rooms hosting patients infected or colonised with carbapenem-resistant *A. baumannii* (CRAB) showed that 91.2% of patients had CRAB detectable in their bed sheets, and 88.2% from the bedrails [129]. Colonised patients show increased environmental contamination than infected (0-106 CFU/cm^2 against 0-29 CFU/cm^2 respectively) [93]. There is no data to support why this might occur and more research is needed, though the current hypotheses are that colonised patients are likely to be less

acutely ill than infected patients, and therefore more mobile, and have more opportunity to interact with their surface environment [93]. Boyce *et al.* (1997) also found that patients with MRSA in wound or urine had greater contamination of the environmental surfaces (85% of samples) within their room compared with patients with MRSA from sputum, blood or conjunctivae, not wound or urine (36% of samples) [128].

These pathogens can be isolated from the environment and matched with current patient strain. Importantly, these pathogens are also being isolated post-terminal clean [130-132]. Eckstein *et al.* (2007) sampled high-touch surfaces within VRE or *C. difficile* positive patient rooms and found that prior to a cleaning training intervention, post-terminal cleaning was poor. N= 72 of the 102 samples taken from VRE-positive patient rooms, after cleaning, N= 58 samples were positive for VRE [133]. While this represents a reduction, it is evidence cleaning is insufficient. For *C. difficile*, prior to cleaning N= 30 of the 54 samples taken were positive, and post-clean, N=24 of 54 samples were positive [133]. This study noted that the cleaning staff were most effective at cleaning the bedrails, while work undertaken later within this thesis sampling a paediatric outpatient unit (section 6.1), found that before cleaning the mean CFU recovered from bed rails were 14.7CFU/plate (N= 18 samples). After cleaning, the average CFU were 15.5 CFU/plate (N= 16 samples) highlighting the variability in cleaning staff performance across different hospital settings and the need for training interventions to be tailored to individual facilities and cleaning teams (section 7.5.3). This recovery post-terminal clean is leaving future occupants at increased risk for acquiring this strain as the

environment is contaminated with this organism, and transmission could occur to the patient from either the patient directly if they are ambulatory, or by healthcare workers [134]. Meaning, current cleaning interventions are not enough and more needs to be done. If hand hygiene is not undertaken before and after every patient interaction, cross-transmission can easily occur from these contaminated surfaces [135, 136] evidenced by the most cases of cross-transmission occurring within areas of higher hand contamination, while enhanced hand hygiene results in a lower incident of HCAI, as documented in studies as far back as 1988 [137, 138].

Additionally, our picture of surface contamination is not complete. Much of the research assessing clinical surfaces is undertaken with traditional microbiological sampling, or with even more rudimentary ATP or UV marker assessments [139-142]. Such fluorescent marking methods for assessing cleaning is a popular method among healthcare professionals trying to assess their clinical spaces, as they represent a fast and cheap method of assessing cleaning [142]. Molecular methods, such as PCR analysis, should be considered in order to give a true analysis of the diversity of species present, as a single test can identify organisms at species and subspecies level, as well as inferring phylogenetic relationships between organisms [143]. Polymerase chain reaction (PCR) is an advanced molecular method of many uses. One of which, includes fast and accurate organism detection [144]. PCR assesses the DNA within a sample and this is referenced against template samples of known organisms. PCR analysis is discussed further within section 8.1. There are many organisms we have yet to discover. New organisms are identified and re-classified all the time; of the greater than 10 million species of microorganisms discovered, only 10,000 have

been cultured in the lab, and only 100,000 have classified sequences. Therefore, the true link between surfaces and HCAI both known and unknown are yet to be revealed. Environmental biologists estimate that just less than 2% of organisms can be cultured in the laboratory environment [145]. This means we are significantly underestimating what organisms and clinically significant isolates are on our surfaces, both inside and outside the clinical space.

1.4.2 HOW PATHOGENS SURVIVE ON SURFACES

Different organisms have varying methods in which to allow persistence and survival on surfaces. Some pathogens are notorious for their ability to survive long-term on surfaces, even under harsh conditions [146]. Table 1.1 outlines the range of survival of different organisms. All the organisms were tested under laboratory conditions except for one of the studies for *C. difficile,* in which a real clinical space was used, though the room was unused. While lab-based studies cannot ever replicate the fluctuating clinical environment, and will not perfectly represent the conditions in a real clinical space, they represent the best possible data for risk assessment, when giving a true idea of how long an organism can survive when subjected to different environmental conditions on different surface types. However, it is important to note that in the real clinical environment, organisms will be exposed to different environmental stressors, cleaning interventions, outbreak and movement of healthcare workers and patients interacting with the surface environment, which will lead to different outcomes as these conditions cannot be replicated . If effective cleaning is not undertaken, these organisms can persist and present a risk to the patient [135, 136, 147].

Table 1.2 Survival of several clinically significant pathogens as taken from Hota 2004 and Kramer 2006 [94, 146].

Pathogen	Survival	Additional Information	Lab based study?
Influenza virus	24-48 hrs	Non-porous surfaces	Yes
Parainfluenza virus	6-10 hrs	6 hrs for clothing, 10 hrs for non-porous surfaces	Yes
Hepatitis B virus	7 days	Environmental contamination within blood (e.g. used lancet)	Yes
SARS-associated coronavirus	24-72 hrs	On fomites	Yes
Candida spp.	3-14 days	On fomites, survival time dependant on species	Yes
C. difficile	5 months	On floors	No - Hospital based (floor of unused room)
A. baumannii	33 days	On plastic	Yes
S. aureus including MRSA	2 days-7 months	2 days on plastic, ≤9 after drying, up to 7 months on dry surfaces	Yes
Enterococcus spp. including VRE and VSE	5 days-4 months	≤58 days On countertops	Yes
Klebsiella spp.	2 hrs >30 months	Dry surfaces	Yes
P. aeruginosa	6 hrs-16 months	On dry surfaces, 5 weeks on dry floors	Yes

This ability to survive long-term in the environment is a result of biofilm. Biofilms are a structured community of microorganisms that are attached to a surface. Biofilms provide a protective 'shell' called the matrix, which allows

organisms in biofilm to not only resist pressure from the environment, but resist cleaning [148]. Biofilms are discussed further in section 3.1. Many organisms have the ability to form biofilm, which allows them to survive well on clinical surfaces, despite these surfaces posing a harsh environment [94, 149]. Biofilms are communities of organisms contained within a self-produced matrix, consisting of extracellular polymeric substances [150]. This matrix creates a protective barrier to allow the organisms to resist desiccation. Bacteria within biofilm are 100-1,000 times more resistant to cleaning than their planktonic counterparts [150], which represents a real concern for hospital cleaning. Regular cleaning prevents the build-up of these biofilms, and established biofilms can be destroyed by exerting firm pressure on the surface when cleaning to allow physical removal with a cloth and detergent, followed by a disinfection step. 'Firm' pressure is used to give physical action and allow the breakdown of biofilm. This exertion of force is critical to effective cleaning, though there is no standard as to what 'firm' pressure might be, when applied person to person. As such, this is a subjective measurement, though it is understood that 'firm' pressure relates to the physical exertion while cleaning. A study by Ledwoch *et al.* (2021) shows that disinfectant treatments (sodium dichloroisocyanurate, peracetic acid, hydrogen peroxide vapour, atmospheric plasma) were not effective in reducing bacterial viability of dry biofilms without a physical removal step, and produced <1 log_{10} reduction in viability, as the mechanical wiping action disrupts the EPS matrix and weakens the biofilm, increasing susceptibility to other cleaning agents [151].

Organisms form biofilm in response to environmental stressors, and there are multiple cues that can trigger biofilm formation, from external factors to internal

production of autoinducers by the bacterial community, molecules which are secreted to cue activation of the metabolic pathways needed for biofilm formation [148, 152]. This is shown in perfect example by the cell to cell communication called quorum sensing, which has been studied well in *P. aeruginosa.* Biofilms are further discussed in relation to cleaning in section 3.1.

1.4.3 How do we know if a surface is safe?

Now there is evidence that organisms have been found on surfaces and can persist well, knowing if a surface can be classified as 'safe' for patients can be difficult. Organism persistence is defined as an epigenetic trait, which allows an organism to survive in unfavorable conditions [147]. There are many bacterial mechanisms which allow organisms to adapt for survival in changing environments, such as sporulation [147]. The current UK guidance requires a surface to reach a visibly clean standard, though this gives no insight into the microbiological safety of a surface. Surfaces can look visibly clean, but still be contaminated with clinically significant pathogens. A study by Ferreira *et al.* (2011) found of 100 assessments of ICU surfaces assessed by visual inspection post-clean, 80% were determined as clean. Under microbiological sampling, just 19% were determined as clean [153]. Griffith *et al.* (2000) found similar results, where 113 surfaces within an operating theatre were sampled. Of these, 82% passed by visual inspection, while only 30% were microbiologically safe [154]. While other methods of surface assessment are not mandated, there are options for taking a closer look at surfaces. These options are; microbiological sampling, ultraviolet (UV) marking before and after cleaning, or adenosine triphosphate (ATP) sampling. Each of these methods have associated costs and produce different results appropriate for

answering different research questions, such as presence or absence of a specific pathogen, or general cleaning efficacy. Microbiological sampling is, arguably, the most complete method of assessing surfaces, as this has the potential to give both qualitative and quantitative assessment of organisms, as well as insight into the species of organism present, not just an idea of presence or absence of contamination. However, microbiological sampling can be expensive, time consuming, and choosing a sampling device and interpreting the results can be challenging, as the current guidance is lacking and confusing [139, 155] and even in heavily-mandated industries, such as the food industry, knowing how to sample various environments is challenging [156]. UV spot marking is a fast, cheap and effective method of allowing a cleaning team to see how their efforts are having an impact on their environment. There are many commercially available brands of UV-marker for use within the clinical space in lotion, powder or gel-dabber form all containing a fluorescent material, though more rudimentary materials can be used. Florescence is the emission of photons from molecules that have been previously brought to an electronically excited quantum state by absorbing light [157]. The key properties needed for a UV marker are; the ability to adhere to hard surfaces, to be invisible to the naked eye after drying on the surface, ability to be detected under UV-light, and that the marker can be cleaned from the surface by wiping with moderate pressure with a dampened cloth or wipe. Less-traditional sources of UV-reactive materials have been explored, from turmeric powder to washing detergent granules [158]. A study by Dewangan and Gaikwad (2020) used an undiluted commercial liquid detergent as a marker for assessing hospital surfaces. This

highlights the flexibility of such systems, and how they can be very low-cost, and represent a great alternative for clinical spaces that want to test surfaces, but do not have the resources for commercially available UV or ATP markers, or other traditional plate-count assessments. The UV-reactive gel or powder marker, which is invisible to the naked eye, is placed prior to cleaning, and surfaces are identified following cleaning using a handheld UV torch, as shown in figure 1.2. Surfaces that have not been cleaned properly, or that have been missed, will reveal the marker under UV light, allowing cleaning teams to visualise these UV markers in terms of a contaminant. These markers are non-toxic and are readily removed with standard cleaning practices [159].

Figure 1.2 UV marker gel on a railing within a clinical space showing failure post-cleaning from a paediatric CICU.

ATP marker systems measure the amount of ATP on a surface. ATP is a nucleotide which is used to carry energy within cells and is present in all known living and active cells [160]. Therefore, greater ATP is associated with more living cells or organisms on a surface, and ATP presence is used as a proxy for organism number

[161]. Increased ATP produces a stronger light reaction within the swab system containing the enzyme luciferase, which is a naturally occurring light-producing enzyme associated with fireflies and luminescent marine life [162]. The swab is read by commercially available ATP swab readers, and this produces a measurement in relative light units (RLU) [161]. The relationship between RLU and how it relates to CFU is under debate, though manufacturers of ATP systems do not explicitly state that RLU correlates with CFU [163-165]. Additionally, presence of organic matter or cleaning agent residues can have an impact on how well the assay works, therefore these swabs are for information only and training purposes for cleaning teams, not for true quantification or risk assessment of clinical surfaces. It is important that ATP swab systems are only used as a training adjunct, and not a replacement for microbiological sampling [140].

1.4.4 Recovery and methods

There are many options for recovering organisms from surfaces, each with their own associated advantages and limitations [166-168]. Environmental monitoring can be broken down into two broad categories of sampling goal; assessing clinical risk or environmental cleanliness. For assessing clinical risk, specific pathogen detection is undertaken, usually with swabs and enrichment to produce highly sensitive results [169], or contact plates with selective agar for less sensitive results [170]. This specific pathogen information is important to assess the ‘clinical risk’ of a surface, which assesses if a surface poses a risk to a patient for acquiring a HCAI, which can be related to how vulnerable the patient is, how critical the surface is and the likelihood of transmission to the patient, and the pathogen risk profile

including antibiotic resistance, mode of transmission and persistence [171]. For general environmental cleanliness, a quantitative assessment is done, usually with contact plates.

1.4.4.1 ENVIRONMENTAL MONITORING

Environmental screening is not currently mandated, and the possible impact of routine screening is debated [172]. Environmental monitoring programmes contain comprehensive guidelines on how to sample surfaces within a specific environment. They contain everything a user might need to know about surface sampling, including when and how to sample, and what sampling devices are appropriate to use [139, 173]. There should also be guidance on the type of result produced, how to read these results, and the actions that should be taken following the result, such as how to escalate a surface sample that has a CFU outside of the suggested guidelines. Environmental monitoring programmes are discussed in detail in section 2.6.

1.4.4.2 HOW TO SAMPLE

Surface sampling devices can be divided into direct or indirect methods. Direct methods take the sample straight from the surface and require no further processing. Indirect methods require extraction of the sample from the sampling device and further processing. There are advantages and limitations to the choice of each method, which are discussed further below.

1.4.4.3 SAMPLE PROCESSING

Traditional microbiological methods involve incubating and growing the organism, usually overnight, then reading them with a plate count method either directly from a sampler (contact plate, dipslide, petrifilm) or after aliquoting a sample

onto an agar plate (swabs, sponges) [174]. Direct contact methods are discussed in more detail in sections 2.4.1.1-2.4.1.3. The CFU are read, and results can be given in CFU/cm^2 or CFU/plate [174]. These methods are generally simple and can give a qualitative or quantitative result. However, the organisms require time to grow, and stressed or damaged cells that do not grow can be underestimated. All traditional microbiological methods have a limited of detection (LOD). This is defined as the lowest concentration (number of colonies) that can be measured with statistical significance [175, 176]. Limit of detection is critical to provide confidence in the ability of a test to accurately detect low levels of contaminants in relation to surface sampling assessing cleaning, as some environmental pathogens only need to be present in very low numbers to be of concern [177]. The infectious dose of *C. difficile* needed to cause clinical infection can be as low as 1 CFU in mouse models [90]. This clinical risk, or risk to patient, is the main concern when determining if hospital surfaces are 'safe' or not, therefore the LOD of a chosen surface sampling method must form a part of this risk assessment when determining if a surface is clean or not.

Additionally, these methods can only recover viable culturable organisms, and there are many organisms that are not recoverable by this method, which are deemed 'unculturable' meaning that, for now, we do not have enough understanding or the capacity to recreate the favorable growth conditions for an individual organism at this time [108].

Molecular methods represent a more sensitive and faster way of processing samples [178]. It is only with these molecular techniques that mixed bacterial communities can be studies in their entirety, without the bias of culture [145].

Polymerase chain reaction (PCR) and quantitative PCR (qPCR) allows amplification of target DNA from a swab sample [178]. However, PCR cannot distinguish between live or dead cells, and cannot determine if a positive result could related to clinical risk, as any present DNA fragment, viable or non-viable, will produce a positive result. This means the type of result a technician is looking for should be carefully considered when looking between molecular or traditional plate-count methods, or a combination of both, as plate count methods give insight into only true viable cells, however the microbial diversity is significantly underestimated. Unlike with PCR, where microbial diversity can be assessed independently of culture methods [179]. If an environment is to be investigated for a single organism, to determine patient risk, such as MRSA, traditional agar based contact plate sampling is an excellent choice. If an environment is to be investigated to determine the possibility of patient-to-fomite transfer of a specific organism strain, or the outbreak origin of a specific strain, then PCR must be used, as traditional plate-count method cannot produce this information.

To run PCR, targeted primers which are the forward and reverse complement of the target sequence, are used to search within the sample for target pathogens or the pathogen of interest [180, 181]. PCR works by running the sample containing the target DNA through a cycle of steps of heating and cooling by a thermocycler machine, to exponentially amplify even the smallest segments of DNA [182]. qPCR works under the same process, and has the ability to quantify one or several DNA targets within the same sample, using optical systems to capture fluorescence which is read by computer software which calculates the emission of fluorescence with the

amount of target DNA in each cycle within the sample [183]. The sample is set up with all the components needed to amplify and copy the target DNA; from DNA polymerase enzymes and forward and reverse primers needed to read and rebuild copies of the target, [181, 182, 184] and nucleotides and buffers [183]. The set up process is detailed in section 8.3.1.6 and the steps are shown in figure 1.3. The cycles run as follows; the thermocycler heats up the sample breaking the hydrogen bonds within the DNA double helix. The thermocycler then cools the sample, allowing the primers to anneal to the complimentary bases on the now broken DNA strands. The next step is extension, where the DNA polymerase is heated quickly and completes the replication of the target. The DNA has now been replicated, and the cycle is repeated for up to 35 cycles [183, 184].

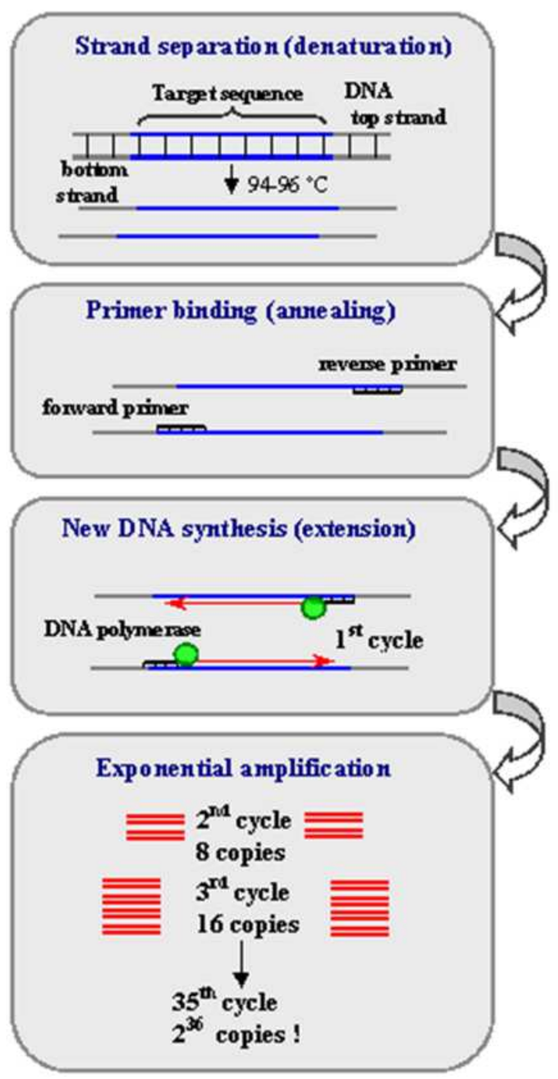


Figure 1.3 Polymerase chain reaction process as taken from National Library of Medicine [185]

PCR has a range of important applications. From clinical diagnostics, and rapid detection of difficult to culture pathogens [183] to detecting a DNA marker as a surrogate for hospital infection, as used within this thesis [186].

This method has the potential for sample pooling, in which many samples can be assessed simultaneously [178]. There is also the option of setting up a multiplex, in which several primer types can be used, which can search a single sample simultaneously for several clinically significant pathogens. Samples do not require

overnight growth, and PCR or qPCR can be undertaken immediately after sampling the surface. Results are usually available within 45 minutes to 1.5 hours, depending on the machine and run type required.

Matrix-assisted laser desorption/ionisation time of flight, or MALDI-TOF, is a sample analysis method that reads the proteins within the sample, and compares this with a large database. In 1996, the first spectral fingerprints of an organism, *Bacillus*, were revealed using MALDI-TOF technique [187]. This method identifies a sample that has been recovered from a surface. MALDI-TOF works by detected mass to charge ratio of the ribosomal proteins within the sample [188]. This provides a unique mass spectrum of the organism, giving a 'fingerprint' of the bacteria characterised by ion peaks [189]. MALDI-TOF can also be used to assess organic molecules such as nucleic acids, proteins and whole microorganisms, though is most commonly known in microbiology [189]. It then provides the closest match between the sample and its reference database and gives very specific detail on the organism and species discovered [189]. The workflow of loading a MALDI-TOF plate is depicted below in figure 1.4.

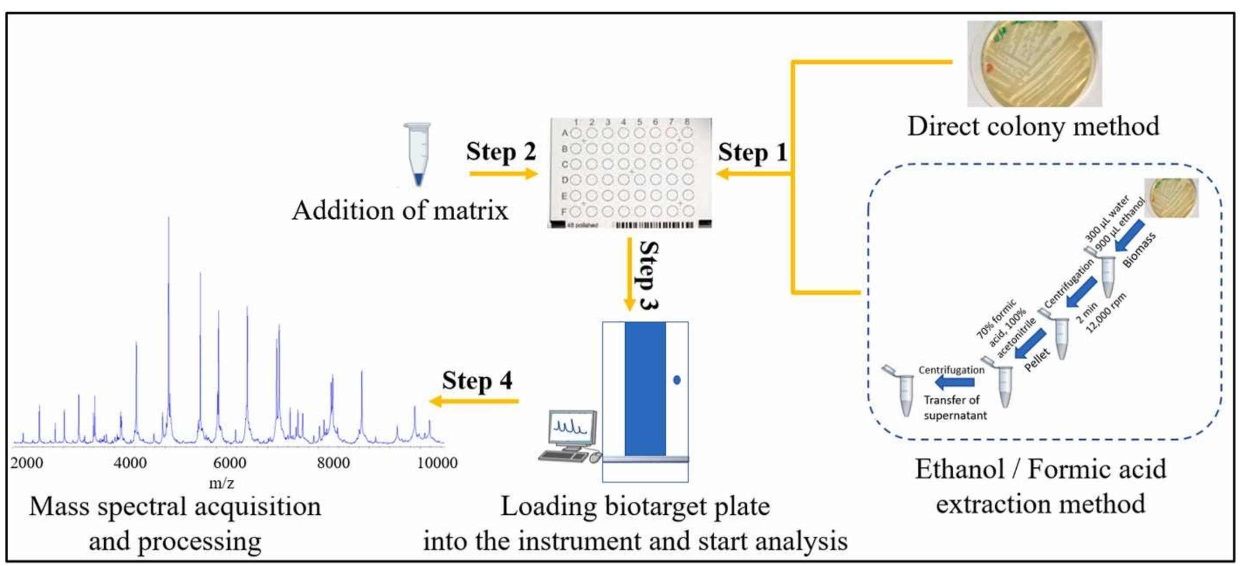


Figure 1.4 Workflow of bacterial identification using the MALDI-TOF method taken from Asfaq et al. 2022 [189].

The organisms must be grown overnight and colonies picked and placed onto a special MALDI-TOF plate to be read as shown in figure 1.4, but once loaded, the result is given quickly compared with traditional microbiological methods [189]. A full 96-sample plate can be loaded read in under 30 minutes, whereas traditional plate count methods take a minimum of 24 hours. While initial set up and purchase of these systems are costly, they remain low cost compared to PCR testing, and faster than traditional microbiological methods [190].

Therefore, molecular methods are generally faster and more sensitive than traditional microbiological methods, though require more maintenance, have higher set up costs, use expensive reagents and will need skilled technicians to use and interpret the results from these machines.

1.5 CLEANING AND BEHAVIOUR

Cleaning is the physical removal of dirt, debris, general soiling and infectious materials. Within this thesis, traditional cleaning relates to the physical action of removing dirt, debris and soiling with a cloth, wipe or mop, with disinfectants of detergents. Novel cleaning methods are the non-physical methods or no-touch systems for cleaning, including ultraviolet light and hydrogen peroxide vapour systems. Cleaning in a hospital is important to keep the surfaces clear from potential pathogens, as well as providing patients with a visibly pleasing environment in which to receive their care. Routine, effective cleaning keeps patients safe.

There are many options for surface cleaning. National guidelines provide instructions on when to use a disinfectant or detergent, and the recommended frequency of cleaning, however there is still a lack of evidence-based guidance on exactly how to undertake this cleaning and clear advice that explores the different types of cleaning agents and technologies available today [191].

Cleaning can be broadly broken down into high level, low level and intermediate, in which the high-level surfaces are deemed to pose the most risk to the patient. High-risk areas must be cleaned with more powerful cleaning agents, ensuring no organisms are left on the surfaces [192]. Cleaning agents can be broken down into traditional liquid cleaning agents, such as quaternary ammonium compounds, chlorines and alcohols, and novel cleaning technologies, such as hydrogen peroxide vapour, ozone and UV-based disinfection devices, all of which are explored in detail in chapter 4. Physical cleaning with detergents followed by high-concentration sodium hypochlorite or hydrogen peroxide vapour systems (HPV) can

work well here. Lower risk surfaces, such as general ward areas, can be cleaned with less powerful agents, and often, good thorough cleaning with detergents can produce good results, which has been defined within the literature as a 5 log_{10} reduction in CFU [193] related to the >5 log_{10} reduction surface cleaning wipes are required to demonstrate by the European Chemicals Agency [194]. Detergents have demonstrated that they can produce a 3-5 log_{10} reduction in surface contamination when tested against different organisms on different surface materials, and that even just water alone with a microfiber cloth could produce a modest 1-2 log_{10} removal [195].

The activities performed in the hospital environment and the housing of infected and colonised patients mean clinical surfaces are routinely exposed to organisms. These surfaces, if not properly and frequently cleaned, can harbour potential pathogens that can contribute to HCAI. Cleaning within hospitals has been found to be lacking [196] which represents a serious issue.

Poor cleaning and low compliance can be caused by a number of issues. Poor cleaning is evidenced well in the literature, and cleaning as a whole is variable [159, 197, 198]. An assessment of 27 ICU centers found that prior to cleaning training interventions delivered across all the hospitals, on average (mean), just 48.1% of the high-touch surfaces were cleaned [199]. Post-training intervention, cleaning improved to an average of 82% of surfaces cleaned [199]. This training is the key to improving cleaning compliance. A multi-center REACH study by Mitchell *et al.* (2019) implemented a cleaning bundle focusing on technique and training with feedback for the staff. Post-intervention, cleaning frequency of surface increased from 64% to 86%

in patient rooms, and VRE infections reduced [197]. Cleaning training is discussed further in section 7.5.2-7.5.4.

Cleaning is reliant on the compliance of an individual, and human behaviour can be difficult to moderate or control. Adhering to protocols and guidelines allows the most effective cleaning to be undertaken. However, human behaviour and individual perception of their job role has a large impact on how effective cleaning can be, and this issue is only worsened by the lack of high quality and frequent cleaning training provided for cleaning staff.

Currently, the quality and quantity of cleaning training varies across hospital settings around the world [191, 200, 201]. Even within the UK, cleaning training is different between NHS trusts. What training consists of and how often training must be undertaken is not currently mandated, and is therefore left to the individual choice of different hospitals. As such, training is often lacking and incomplete, and re-training and competency tests are not undertaken. To worsen this issue, the differences between in-house and outsourced cleaning staff has led to a combination of poor training, varied training and staff members feeling isolated and not valued as part of a team. This demotivation can have an impact on how well these staff members can do their job.

Domestic workers can only clean to the best of their ability if they are trained appropriately, and with enough frequency that allows consistent refreshing of core cleaning principles. Often, domestic workers are considered ‘low skilled’ and are taught as such. They are not given the basic principles of infection control, which

would provide an important foundation to the rest of their cleaning training. It is critical for a worker to know why they are cleaning in such a way, as opposed to just being trained how to undertake the physical cleaning. Furthermore, learning styles vary between individuals. A training package that may work effectively for some staff, may not be as effective for others. Therefore, focused and evidence-based training is critical to ensure cleaning staff are confident and knowledgeable about their role, and aware of the wider impact they have on the hospital and the safety of the patients [200].

The literature has shown that while great efforts have been made to reduce HCAI [202], HCAI still represents a significant threat and in Europe hospital prevalence of HCAI can be up to 9.3% [203]. The hospital surface environment shows that not only can pathogens survive in the environment [146] but that they are linked to HCAI [12, 204, 205]. Typing studies are linking patent isolates with environmental isolates and providing evidence that the surface environment, previously forgotten, is playing a role in HCAI. However, the exact transmission routes of HCAI can be difficult to determine in busy ward environments. Knowing how to assess the hospital surface environment can be difficult. There is no mandated environmental monitoring within the clinical environment outside of outbreak, so advocating for such measures needs more evidence to further provide evidence-based interventions.

The cause of environmental reservoirs is poor cleaning. Gram-positive and Gram-negative organisms have strategies to survive on surfaces. Some organisms, such as *S. aureus*, is highly resistant to desiccation and can survive long term on

surfaces. Effective, frequent cleaning of surfaces is the key to preventing this. Comprehensive environmental screening with optimal methods will allow healthcare professionals to assess their surfaces, identify trends and to determine if their surfaces are clean. However, cleaning is a complex issue. The literature shows that cleaning training is sporadic and lacking within the clinical environment. There is a lack of respect and understanding for the vital role cleaners play in the hospital environment. Cleaning training needs to be delivered in order to improve cleaning. Improved cleaning can reduce the bioburden of the surface environment, which has been linked to reduced HCAI. Knowing how to undertake this training must be assessed, as multifaceted and large-scale interventions are not appropriate for all hospital settings.

Based on these concerns, this thesis seeks to assess the hospital surface environment. To understand what pathogens may be present on surfaces, how to find and sample such pathogens, then how to prevent these pathogens from persisting on surfaces by cleaning. To explore cleaning in relation to the options for environmental surfaces, how different cleaning agents perform, and what healthcare professionals may choose to sample their surfaces with either liquid or wipe-based cleaning agents. As this cleaning step is vital, but it has been shown that cleaning compliance is not as effective as it should be, cleaning efficacy must be investigated to determine what makes a complete cleaning training programme, and how might cleaning training be effectively implemented even in busy wards with lower resources, and what other factors may play a role in how well cleaning is undertaken, assessing the ‘human element’ of cleaning. In relation to cleaning training, cleaning

protocols, movement of an infectious agent can provide insights into how a surrogate pathogen will truly move around the clinical space, and what this movement may mean for cleaning training and intervention.

Chapter 2 UNDERSTANDING SURFACE SAMPLING WITHIN THE CLINICAL ENVIRONMENT.

2.1 INTRODUCTION

Healthcare associated infections (HCAIs) represent an ever-growing burden to society, both in the form of loss of life and financial consequences due to increased use of resources and additional bed days. It is estimated that HCAIs lead to a loss of up to $147 billion a year in the US alone [206] and in the UK, it is believed the annual financial cost of HCAI is £1 billion [207]. Acquisition of a healthcare-associated infection can lead to increased morbidity and mortality, while putting an additional strain on antimicrobial stewardship Infection transmission between healthcare workers and patients is often considered, mostly, in terms of hand hygiene and the risk hands pose to the patient. Surfaces, however, also have an important role to play in the transmission of infection [208, 209].

It is proven that not only can pathogens survive and persist on surfaces (section 1.4.2, [90, 208], but that surfaces are a critical component in infection transmission [208]. As the surface environment plays such a critical role in HCAI, it is important to assess the environment and build a picture of the microbiome of individual surface environments [210, 211]. Knowledge of the normal background constituents of specific environments, outside of outbreak scenario, can help identify important changes in organism type or quantity, which could indicate poor cleaning, increased risk of transmission and HCAI, while also helping to identify potential sentinel sites and known reservoirs for infection. Knowing these sites can be critical to identifying sources during outbreak and provide a focused set of sites to sample

when looking for specific outbreak organisms. Knowing where the contamination is in the environment, what organisms make up each specific clinical environment and identification of reservoirs is critical. As such, development of an effective environmental monitoring programme is important. Monitoring the clinical surface environment can be in the form of specific pathogen detection, or general sampling to monitor cleaning efficacy [170].

Environmental monitoring, both microbiological (plate count) and non-microbiological (ATP testing), is mandated in both the food and pharmaceutical industry [139, 156]. Both critical and non-critical environments are sampled in order to assess the potential risk to a product or consumer. These sampling guidelines are produced for each site as per strict guidelines in the Orange Guide, United States Pharmacopeia (USP 1116) and ISO standards (ISO 14644, ISO 18593). Despite rigorous and thorough sampling documentation in these industries, no such guidelines exist for the clinical environment, and surface sampling is not mandated. While the multifactorial nature of clinical environments make the production of guidance difficult, unlike the heavily regulated and largely homogenous food and pharmaceutical settings, this need must still be met.

Currently, for the clinical environment, there are only two proposed contamination cut-off standards to determine if a surface is ‘safe’ or ‘unsafe’, classified by Dancer [212] and Griffith [213]. While an important contribution, these proposed guidelines are not evidenced-based, and are still lacking the wide variety of information needed to build an environmental monitoring programme. As such, healthcare professionals seeking to sample their environment are left without the

tools and evidence-based guidelines on how and when to sample their surfaces, and how to process and interpret their results.

Many of the surface sampling studies recovered came from food and pharmaceutical industries. This is, perhaps, unsurprising as surface sampling is heavily mandated within these industries, and is a requirement for all manufacturing and food testing laboratories. These industries should be considered more closely, and used as a potential guideline for building effective environmental monitoring programmes for clinical environments, as well as assessing the efficacy of different sampling devices. While direct application of these programmes is not appropriate for clinical settings, they represent a well-established baseline or template for future adaptation. Petrifilms and dipslides were two sampling devices that are used often in the food and pharmaceutical industry, for water and environmental surface and air sampling. These devices should also be considered for their applications to the clinical environment. This is one example of how there are many potentially useful guidelines and sampling devices currently used within other industries that could be applied to sampling within the clinical environment.

With the understanding that the hospital surface environment can be a reservoir for clinically significant pathogens, and that this plays a role in the transmission of HCAI, it can be said that monitoring such surfaces for both clinical risk and cleaning efficacy is important. Building an idea of what surface sampling devices are available and how they might relate to use within the clinical space is of value. Ideally, the current data across different industries and different surface sampling devices could produce a detailed meta-analysis to produce a simple user-

guide for healthcare professionals wishing to sample their surfaces. To attempt to fill this gap, a literature review assessing sampling devices was undertaken. The literature revealed a wide range of surface sampling devices that could be suitable for the clinical environment. The nature of the literature revealed how different method choices and testing conditions led to a multitude of efficacies reported for different devices. However, the available evidence was sporadic and lacking consistency and completeness. Due to the difficulty in drawing conclusions between the literature and the lack of available guidelines, healthcare professionals seeking to sample their environment face a wide range of contradictory, difficult to read, and often unhelpful information. Compiling the available literature in a format suitable for healthcare professionals allows evidence-based informed decisions to be made on how to sample different surfaces.

A gap analysis of the literature identified several confounding factors and gaps in the evidence. The literature indicated how a large range of factors could change how effective different sampling devices are, such as target organism, surface material, surface bioburden and the presence of residual cleaning agents on the surfaces. Despite these factors causing change in recoveries, different studies used a wide range of methods and data interpretation methods. As such, studies were not comparable. Furthermore, due to the lack of publications, many organisms were not tested under different sampling conditions, leaving large gaps in the literature.

2.2 RESEARCH AIMS

The aim of this chapter was to perform a literature review and collate information from all evidence-based studies which used microbiological surface sampling in order to improve the current understanding of surface sampling methods. The review of the evidence allowed some conclusions to be drawn, but also highlighted the many gaps present. This gap analysis will inform further work undertaken in chapter 3.

The factors involved with surface sampling were explored and discussed as follows:

1. Range of different surface sampling devices available (contact plates, dipslides, petrifilms, swabs, and sponges).
2. Sampling for different organisms and the impact on recovery.
3. How to process samples, the types of results produced, and associated limitations of different methods.
4. The role of environmental factors and the impact on surface sampling, such as stressed and damaged cells, adsorbed cells, wet or dry surfaces, and surface material and topography.
5. How to implement these into an effective environmental monitoring programme.

The results from this chapter were published as a sampling guide in the Journal of Hospital Infection; How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence [139]. These results were also used to inform the following publication; Who decides what's relevant? Factors driving publication on clinically significant organisms [23] in Infection Prevention in Practice.

2.3 METHODS

Due to the wide range of sampling devices, sampling settings, and inclusion of several disciplines, a traditional systematic review of surface sampling devices was not suitable. Instead, a semi-systematic review procedure was adopted. Systematic review procedure was followed as closely as possible, though could not fall within the PRISMA guidelines [214]. The following online repositories were searched; ScienceDirect, Web of Science and Medline (PubMed). The keywords included; hospital, environment, sampling, surface, monitoring, contamination, swab, sponge, petrifilm, dipslide and contact plate. Exact breakdown of search terms can be found in Appendix. The inclusion exclusion criteria are as listed below;

Table 2.1 Inclusion and exclusion criteria for sampling device literature review (Rawlinson, Ciric *et al*. (2019). [139]

Topic	Inclusion criteria	Exclusion criteria
Language	English	Non-English
Date	All studies published up until March 2019	n/a
Organisms	All organisms found in the hospital environment; bacterial, fungal, and viral	None
Surfaces	Walls, floors, medical equipment, all high-touch surfaces, furniture	Hand hygiene, invasive medical devices, air, water samples
Literature	All data-based studies that appeared in peer-reviewed journals, with no limitation on publication date	Non-peer-reviewed sources, theses, dissertations and presentations
Methodology	Comprehensive methodology with multiple or strong sampling techniques	Poor methodology, surface area not defined, pass/fail criteria not defined
Content	Data-based studies	Opinion studies, studies with no methods and results
Study focus	Background environmental monitoring, general monitoring, specific pathogen monitoring	Outbreak and cleaning intervention studies that could skew results
Location	Hospitals worldwide, regardless of specialty and patient subset, lab based food and pharmaceutical industry studies	Dental surgeries, GP clinics, samples taken in hospital laboratories only

2.4 RESULTS

The review of the literature identified N=73 studies fulfilling the criteria. Of which, N= 13 were sampling studies undertaken within the hospital. N= 32 were laboratory based using surrogate surfaces. N= 6 different surface sampling devices were identified from the search.

Table 2.2 Studies recovered from the literature review for each surface sampling device

	Colony counting and phenotypic identification	Molecular biology methods used for identification	Total
Contact plate	9 studies [93, 123, 124, 127, 215-219]	0 studies	9 studies
Dipslide	2 studies [123, 126]	0 studies	2 studies
Petrifilm & wipe	2 studies [220, 221]	1 study [222]	3 studies
Swab	36 studies [124, 128, 166, 216, 217, 219, 223-252]	16 studies [128, 180, 222, 226, 231, 232, 239, 242-246, 253-256]	52 studies
Sponge	5 studies [129, 229, 232, 257, 258]	2 studies [129, 232]	7 studies
Total	54 studies	19 studies	

2.4.1 SAMPLING DEVICES

The literature review revealed that there are many methods available for surface sampling, varying in cost and ease of use. Different sampling techniques require varying levels of time to use and training for result interpretation. Figure 2.1 below shows the breakdown of studies assessing the different devices. Some methods are more effective than others when recovering from different types of surfaces and for different types of organism. Surface sampling methods can be broadly divided into two categories:

- Direct contact methods, which are pressed directly onto the surface to recover organisms and are then incubated.
- Extraction methods, in which following recovery of organisms from a surface, further processing is needed.

Generally, direct contact methods allow faster sampling and require less training to use. Often they are self-contained and can be directly incubated following sampling. Extraction methods require the additional step to remove the organisms from the sampling device, such as a sponge or a swab. It is critical that the methods are optimised to allow maximum recovery, as there is risk of sample loss, as not all organisms can be effectively recovered from the device, leading to potential false-negative results. However, extraction methods have a wider variety of processing options, as well as enrichment, which can be the difference between recovery and non-recovery of a stressed or damaged organism. Enrichment is the process whereby specific organisms of interest are given ideal conditions to grow in, allowing stressed or damaged cells to also grow in an environment where they do not have to compete

with other species [259]. The enrichment media will carefully match and support the growth and physiological conditions preferred by the organisms of interest [259].

The literature review revealed the most popular surface sampling choices were swabs and contact plates, likely due to healthcare facilities pragmatically selecting these surface sampling devices that are readily available within their environment. Petrifilms, a paper-based sampling device more commonly used in the food and water testing sector and not yet validated for healthcare environments, were the least frequently used.

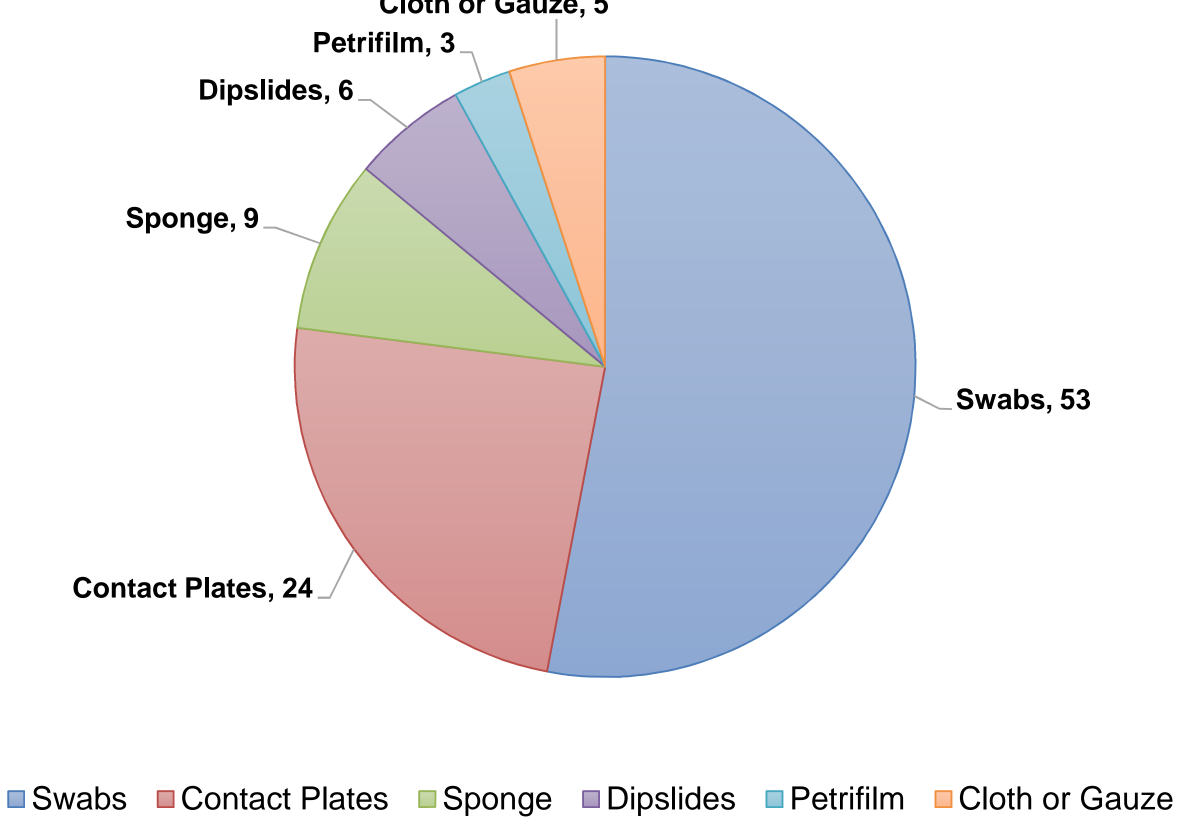
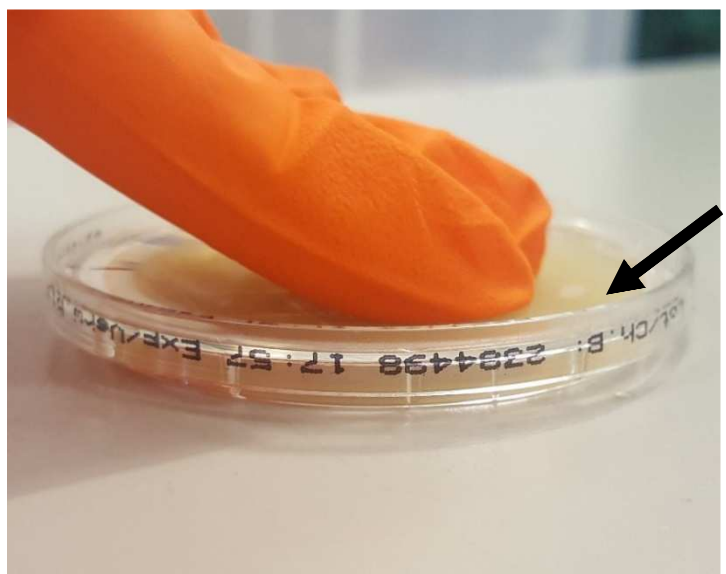


Figure 2.1 Distribution of surface sampling devices used in studies retrieved from the literature review.

2.4.1.1 CONTACT PLATES

Contact plates are convex discs of agar contained within a petri dish. These plates can be made of a range of different media, either selective or non-selective, to aid in the recovery of different types of pathogens [139, 260]. Selective media is a media that encourages only the growth of a specific target organisms by using ingredients that promotes growth of the target organism and eliminate the growth of any other organisms [261]. Antibiotics, salts, dyes are all common additives to produce a selective effect [261]. An advantage of contact plates is the ability to add or purchase them containing a neutralising agent, to prevent residual surface cleaning agents or biocides interfering with organism recovery. Neutralisers can allow surface sampling devices to perform significantly better [139, 262] by neutralising common surface cleaners to allow better pathogen growth and therefore recovery [263-265].



The exposed agar base of the contact plate is pressed with firm pressure against the surface to take a sample. Figure shows gloved hands exerting pressure upon the sampling device to recover sample from the surface.

Figure 2.2 A TSA contact plate taking a surface sample.

To take a sample, the lid is removed and the plate is pressed, agar side down, to the test surface [139, 260]. Firm pressure is given for 10 seconds, at a suggested

force of 25g/cm^2 [266] which represents the balance between user practicality and sampling speed with potential recovery [267]. The lid is then replaced and the plate is incubated as per the time and temperature required for different organism recovery. Following incubation, colony forming units (CFU) can be read directly from the plate and number of organisms quantified [266]. The review revealed, of all surface sampling, 24% used contact plates (table 2.2) used most frequently to sample patient over-bed tables and bed rails, which are both smooth and non-porous surfaces. As a direct method, requiring no further post-test processing, such as is required with swabs, contact plates can often recover more organisms [139, 266]. They were found to be more effective than swabs in recovering organisms from fabric, [268] and methicillin-containing contact plates outperformed both dipslides and swabs when recovering methicillin-resistant *Staphylococcus aureus* (MRSA) from stainless steel surfaces [269, 270]. Overall, contact plates were most effective in recovering *S. aureus* from non-porous surfaces [271].

The advantages of contact plates are as follows;

- Easy and fast to use, with little training required
- Availability in clinical settings as used in pharmacology monitoring
- Reproducible surface area between technicians
- Choice of selective or non-selective agars
- Choice of addition of neutralisers
- Enclosed method; no processing losses
- Quantification by colony counting

- Works well for adsorbed cells

The limitations are as follows;

- Surfaces must be flat and even
- Pressure is variable between technicians
- Does not work well on heavily contaminated surfaces due to clumping of cells, as overgrowth makes enumeration difficult
- Less sensitive than swabs (on mattress surface)
- No enrichment process for stressed or damaged cells to allow extra recovery
- Recovery variabilities between brand

2.4.1.2 DIPSLIDES

Dipslides work similarly to contact plates, and contain two sides of agar, providing two sampling surfaces per device [139, 266]. The agar type is customisable, and each side can contain a different type of growth medium, which could allow both a selective and non-selective sample taken using the same sampling device. Dipslides have the added feature of a flexible shaft, which allows them to sample uneven surfaces in which contact plates would be inappropriate [262]. The sampling handle also allows easy sampling and prevents any contact and contamination of the sampling surface [262].

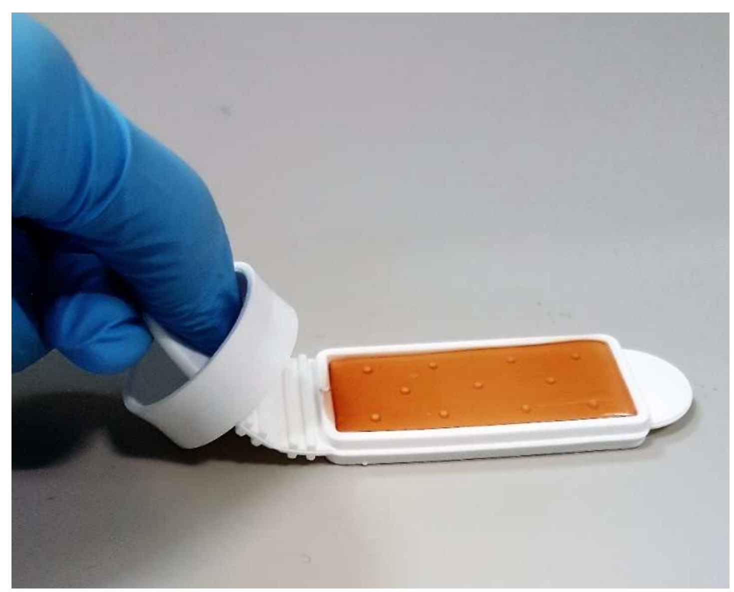


Figure 2.3 Use of a dipslide with selective agar to take a surface sample.

To take a sample, the lid is removed and the agar surface pressed for 10 seconds, with firm pressure, to the surface. 10 seconds is the consistently recommended time as per manufacturer guidelines (Oxoid, Cherwell), though deviations from this, such as sampling fabrics with a 3 second contact time, have been shown [268] Following the sample, the lid is replaced and the sample incubated as per time and temperature required for different organisms. CFU can be read from the plate [262].

Despite showing good promise as an effective sampling device, they were only used in 6% of studies assessed. Dipslides are more often used within the food testing industry, both for water testing and environmental sampling, and they have yet to be considered or validated for clinical surfaces [262]. Dipslides represent a key sampling option within private laboratories as they can be used for both surface and water testing, making them an economical option when a lab frequently tests both

water and surfaces. Additionally, they can be used off site for environmental sampling for water, without keeping multiple types of sampling devices within stock. As food and pharmaceutical industries require consistent and rigorous monitoring of their environments, it is unsurprising that in response to these requirements, other sampling options have been manufacturer tailored specifically for use in these environments that can be validated to require industry standards like those accredited by International Organisation for Standardisation (see section 2.6), like dipslides, as there is a strong need and profit-based market for such devices.

Dipslides were found to perform better than TSA contact plates [262]. They were also found to be better for faecal indicator species recovery when tested against contact plates [262]. It has also been suggested that dipslides represent a safer alternative to contact plates as the cap is more secure, and less likely to come loose during incubation which can cause the agar to dry and cause the test to be unreadable [262].

The advantages of dipslides are as follows;

- Similar to contact plates
- Increased flexibility allowing sampling of uneven surfaces
- Two sides for different samples or an easy replicate of a single sample. Sides can have the same or different customised selective or non-selective agar, allowing personalisation according to function
- Multifunctional; can be used for liquid testing or inoculation following swabbing

- Simple basic analysis available using percentage coverage charts to give fast estimation of CFU/cm^2 surface contamination
- Dipslide comparator app for basic analysis

The limitations are as follows;

- Same as contact plates
- If using the simple percentage coverage chart results are an estimate only
- Lack of routine availability in clinical environments, as they are not used within the clinical space unlike contact plates, that are often used to take samples within the pharmacy preparation areas. Would require special ordering for use

2.4.1.3 **PETRIFILMS**

Petrifilms are a more compact, paper counterpart of contact plates [270]. They must be prepared prior to use by wetting. Petrifilms are impregnated with various dried media, which is reconstituted upon wetting. This allows petrifilms to perform as general count or specific pathogen detection using either selective or non-selective agars [272]. Petrifilms, having a paper surface, have added flexibility and can be wrapped around an uneven or difficult to sample surface, such as a door handle [270].

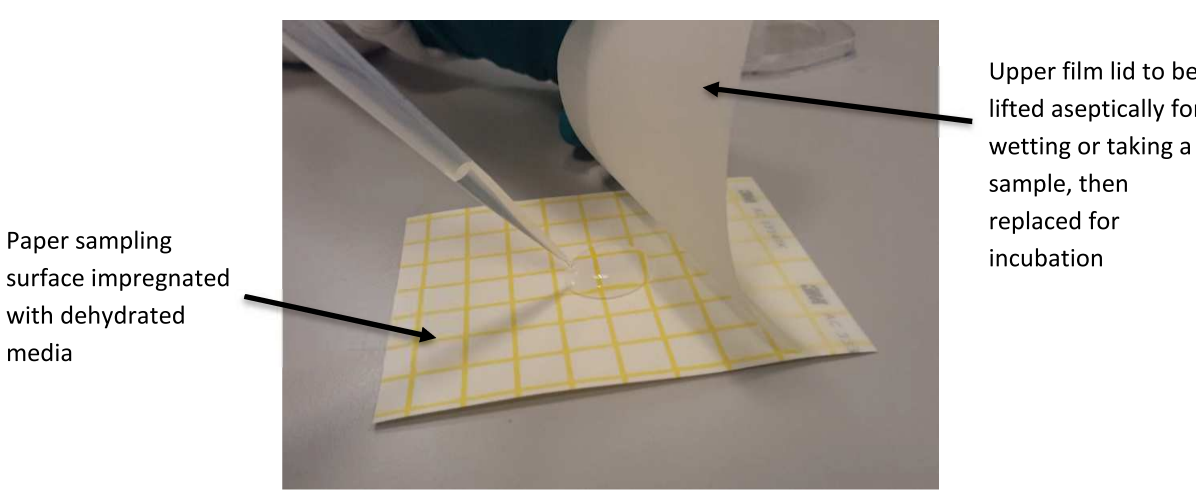


Figure 2.4 Preparation of a petrifilm by wetting the sampling surface.

Petrifilms are purchased dry and must be rehydrated prior to use. To rehydrate, the film surface (shown in figure 2.4 by arrow) is lifted and 1ml of sterile diluted pipetted into the center of the grid-marked paper sampling surface. The upper film lid is then replaced. To allow the dehydrated media to form a classic circle shaped sampling surface, a plastic spreader (provided with all petrifilms from the manufacturer) is pressed against the top of the surface to manipulate the diluent into

a circle. The petrifilm is refrigerated and allowed to set for a minimum of 1 day prior to use to allow the rehydrated gel-media into a circular sampling surface.

Following this refrigeration period, the sampler is ready to use. The upper film is lifted to expose the gel media surface. This gel is pressed against a surface following the same method as a contact plate. Once the sample has been taken, the film is replaced as a 'lid' and the petrifilm is incubated as per time and temperature required for individual organisms.

Petrifilms are often overlooked as a sampling device, as their main use is for passive air sampling. Despite this, petrifilms work well for surface sampling, and have been validated for use in the food industry. Only 3% of studies assessed petrifilms. They were found to be the best sampling method for recovering MRSA from linoleum, mattress, coated steel, and polypropylene, [270] and their flexibility gave them an advantage over contact plates [270].

The advantages of petrifilms are as follows;

- Cheap and easy to use with little training required
- Manufacturer suggest their implementation leads to increased technician efficiency, as petrifilms can be prepared in advance, can be used for both active and passive sampling flexibly, and take up less room in incubators than other, agar-based samplers
- Uses less space during incubation, which is useful when space is limited or many samples need to be taken
- Can be infused with different agars for specific pathogen growth

- Appropriate for both aerobic and anaerobic colony growth
- Appropriate as inoculation plates following swab or sponge method, passive air sampling and finger dabs
- Can be manipulated around uneven sampling sites such as door handles and table edges
- Colonies can be isolated similarly to traditional plates
- Reduced waste and disposal costs
- Plates can be hydrated and stored prior to use, for up to 14 days for Aerobic Colony Counts (ACC)

The limitations of petrifilms are as follows;

- Requires wetting before use with sterile solution such as water, buffered peptone water or saline. This adds an additional risk of outside contamination if the water is not properly sterilised or good aseptic technique is not used when preparing the petrifilm
- Cannot be used for immediate responsive sampling due to the preparation process
- Preparation requirements may make larger sampling numbers impractical
- Less robust in design compared to contact plates and dipslides
- Not validated (industry standard method that has been analysed and determined as fit for purpose and that reported results are true) for any industry other than the food industry
- Not readily available in clinical environments

- Does not work well on heavily contaminated surfaces due to clumping of cells and overgrowth makes enumeration difficult
- Once opened, they must be kept in the refrigerator or frozen
- Plates must be hydrated and kept in the fridge for a minimum of 3 days before use as a contact method

2.4.1.4 SWABS

Swabs are sampling devices that have a plastic or wooden shaft, allowing easy surface sampling without contaminating the collection tip. The tip can be made of various materials, which allows the recovery of different organisms from different surface types. From the literature review, studies assessing the efficacy of different surface sampling devices by challenging the swab against common surfaces like plastic, stainless steel and linoleum in lab settings, it was found that swabs were made of either cotton [177, 273], rayon [168, 270, 274], polyester [168], flocked nylon [270, 274, 275] or macrofoam [168, 276]. Swabs can either be loose, individually wrapped, or self-contained within a collection tube, which can either be dry, or containing a transport or collection fluid.

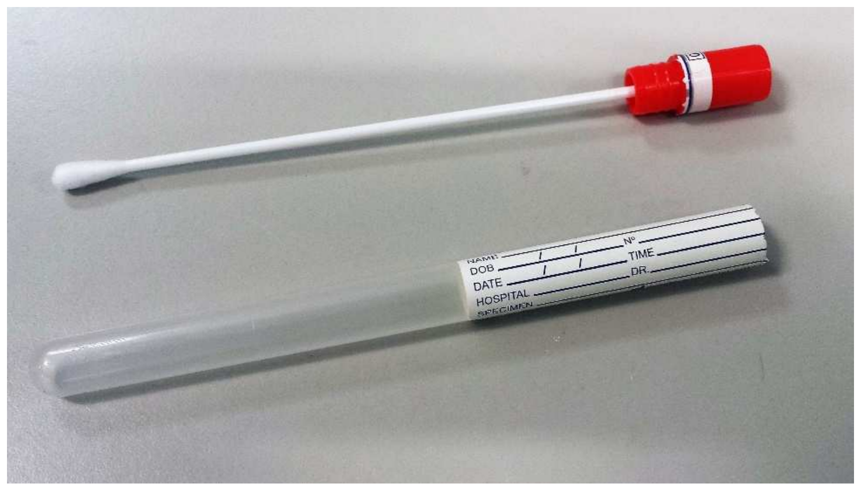


Figure 2.5 Plastic-handled swab with plastic transport tube.

When taken swab samples, it is critical to ensure correct sampling protocol is adhered to. Incorrect sampling can lead to poor recovery, and skew results. Prior to sampling, the swab tip must be wetted in a sterile solution, such as water. When swabbing, enough pressure must be exerted to ensure flex of the shaft. If flex is not apparent, not enough pressure has been applied. Samples are taken in a

10cmx10cm^2 square (or as representative of this size as possible across uneven or smaller surfaces, such as taps and drains) as per manufacturer instructions, with firm horizontal strokes, while rotating the swab tip continuously. This is followed in the same fashion with vertical and diagonal strokes. Post-sampling, the swab is either replaced in its collection tube for microbiological processing, or the tip snapped into a pre-prepared microcentrifuge tube, containing sterile molecular water for molecular processing.

From the reviewed literature, swabs were the most frequently used sampling device, used in 53% of sampling studies. This is potentially due to their availability in the clinical environment, simplicity, and ease of use. Swabs are also useful for taking samples from uneven surfaces or small crevices, such as inside or around tap fittings, in which using other sampling devices would prove difficult due to their inflexibility or size, such as contact plates. Furthermore, swabs are versatile and allow both microbiological or molecular processing. It was found that of all swab types including flocked, macrofoam, cotton, rayon and polyester, macrofoam swabs were found to be the most effective [168, 276]. Swabs with neutralizing buffer were more sensitive than saline-moistened cotton swabs, with sensitivities of 2.6×10^1 MRSA/cm^2 and 2.8×10^2 MRSA/cm^2 respectively for *S. aureus* [170]. Despite these swabs being effective, overall, swabs were difficult to standardise and different users apply different pressure, sample slightly different sizes of surface, use different sampling angles and vary the amount of tip rotation during sampling. For *S. aureus,* variation in recoveries with swabs has been shown to vary between 22-58% [274].

The advantages of swabs are listed as follows;

- Can sample uneven surfaces and crevices
- Easy to exert pressure on surface and recover organisms from biofilm
- Cheap and often readily available within the hospital environment
- Choosing non-sterile loose swabs that can be packaged and autoclaved in-house can further reduce resource costs
- Wide choice of wetting agent and transport medium for method optimisation
- Can use direct inoculation or enrichment methods for increased sensitivity
- Can be processed using molecular methods

The limitations of swabs are as follows;

- Processing losses and variability in recovery following processing choices
- Variable sampling pressure and tip rotation between technicians can produce variable results
- Variable surface area unless sterile guideline coupon is used
- Cost of further processing to remove sample from swab
- Skill required for results interpretation, for microbiological analysis or molecular analysis
- Failure to wet the swab tip significantly reduces recovery

2.4.1.5 **SPONGES**

Sponges are supplied individually or as a sponge-stick device, either dry or pre-moistened. Sponge-stick devices can be easily aseptically removed from their packaging. The long plastic handle ensures no contact with the sponge sampling tip. As sponges are malleable, they can easily be manipulated around uneven surfaces, such as taps and sink areas. The sponge is also significantly larger than a swab tip, and therefore could allow easy sampling of large surface areas, such as in bathrooms.

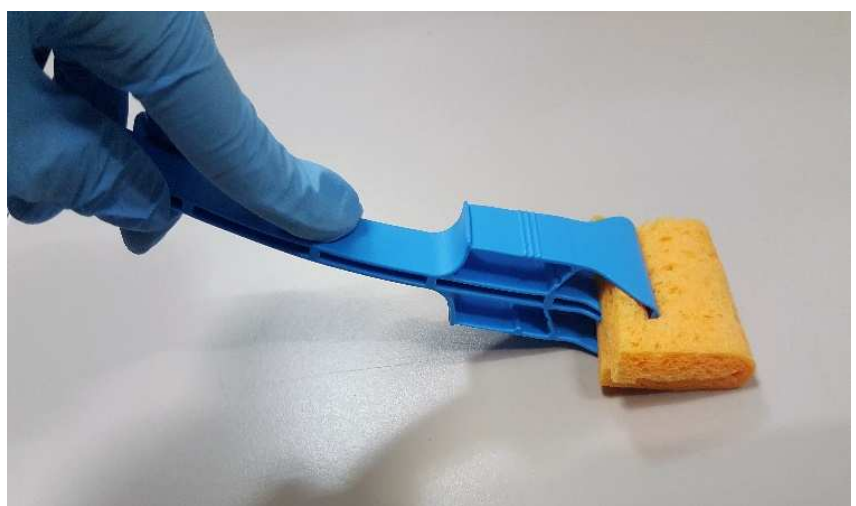


Figure 2.6 Swab stick sampling device in use

To take a sample, the sponge is removed by touching only the handle of the sampling device. The sample is taken similarly to swab samples, by taking a 10x10cm^2 to remain consistent with swab samplers, while exerting firm pressure on the sponge tip allowing flex in the sampling handle. Samples are taken horizontally, vertically, and the two edges of the sponge are used to take the diagonal samples. The sponge is released back into the collection back by squeezing the sides of the sampling stick. The plastic stick is discarded, and the sponge is ready for further processing.

The review revealed sponge devices were not popular with just 9% use. The literature revealed sponges were significantly ($P<0.0001$) better for recovering

Clostridioides difficile than swabs [277]. It has been suggested that sponges work particularly well for *C. difficile* due to the pores within the sponge picking up the spores easily, and the ability of the device to sample a larger area more easily [278] which is advantageous as larger surface area samples were more likely to be positive for *C. difficile* [279]. Sponges were also suggested as potentially more effective for fabric surfaces for some organisms [167]. While sponge-stick devices allow easy sampling, it is important to note that using simple sponges for sampling introduces added difficulty in processing, and excellent aseptic technique must be used during sample collection and handling to avoid contamination and false-positives.

The advantages of sponges are listed below;

- Can sample large surfaces quickly and easily
- Simple sponges are cheap
- Can be manipulated around uneven surfaces
- Literature suggests sponge sampling methods are the most effective way to recover *C. difficile* from surfaces
- Can be used with enrichment methods to increase potential recovery of stressed organisms

The limitations of sponges are as follows;

- Lack of routine availability in the clinical setting
- Potential technician contamination with the loose sponges
- Cost increases for the sponge-stick devices and addition of transport medium, neutralisers and/or sterile collection bags

- Unavoidable post-test processing losses as the sample must be removed from the sponge. There will always be residual colonies trapped within the sampling sponge that cannot be retrieved

2.4.2 OVERALL CONCLUSIONS FOR SAMPLING DEVICES

Comparisons between the literature were difficult, though the following recommendations could be made;

Table 2.3 Suitability of different surface devices as per sampling conditions (Rawlinson, Ciric *et al.* (2019) [139].

	Swab	Contact Plate	Sponge	Dipslide	Petrifilm	References
Wet Surface	+*				+	[177, 209, 263]
Dry Surface		+			+	
Flat Surface		+	+	+		[167, 168, 209, 218, 267, 268, 270, 280]
Uneven Surface	+	-	+	+	+	
High Bioburden	+	-				[170, 177, 274, 280, 281]
Low Bioburden		+	+	+	+	
Injured Cells	+		+			[218, 269, 271, 272, 282]
MRSA		+			+	[170, 270, 275]
S. aureus		+			+	[262, 267, 270]
C. difficile			+			[122]
Gram-negative bacteria	+					[170, 262, 270]
Viruses	+	-	-	-	-	[276]

*cotton, rayon, polyester or macrofoam. Brush-textured swabs perform poorly on wet surfaces.

Empty cells indicate lack of data

The following tentative recommendations on individual surface sampling devices could be made;

- For recovering *S. aureus* from stainless steel, **MRSA-selective contact plates** performed better than dipslides and swabs, and were better overall for non-porous surfaces.
- However, for *P. aeruginosa* and *Salmonella abony,* **macrofoam swabs** worked better than contact plates for recovery from stainless steel.
- Overall, **dipslides** performed very well and should be investigated further as a surface sampling device for the clinical environment.
- Under some conditions, **macrofoam swabs** performed better than other swab types.
- **Sponges** should always be chosen when trying to recover *C. difficile*.
- **TSA contact plates** work well for *Acinetobacter* and *Pseudomonas* spp. recovery when compared to dipslides.

2.4.2.1 TARGET ORGANISM

Target organism causes variance in the effectiveness of each method [275] [270] [268] [262] [267] [274] and regardless of method chosen, recoveries naturally vary between organisms and strain [272] [283] as some organisms will always have higher recovery than others. This is put in example, where *S. aureus* repeatedly gives higher recoveries, regardless of sampling method, than *Staphylococcus epidermidis* [267] and when comparing sampling methods across all the literature, this natural variation is an important consideration.

Even if organisms are subjected to the exact same surface conditions, there is still variation (see section 3.4.2). This is explained by the structure of the organisms. *S. aureus,* a Gram-positive bacteria, is known for its ability to resist desiccation [284]. This is an important factor for environmental survival, and accounts for one of the reasons why Gram-positive organisms survive longer on environmental surfaces compared with Gram-negatives, due to Gram-positive organisms having thicker peptidoglycan to protect them from desiccation [285, 286].This desiccation, or drying out, may mean organisms are not recoverable by traditional plate-count methods, as dead organisms cannot be recovered and stressed organisms may fail to grow. These stressed or dead organisms would only be recovered by molecular methods, see section 1.4.4.3. As organisms have different structures that allow them to attach to surfaces, some organisms are more difficult to recover from a surface (see section 3.1). Difference between organisms can be seen in range of recoveries where *S. aureus* is reported as 46.30-105.26%, whereas *Escherichia coli* has a lower range of between 24.28-80.30% [263]. Furthermore, in a study testing recovery of *Listeria monocytogenes,* it is found that uninjured *L. monocytogenes* gives greater recovery than sub-lethally injured, regardless of sampling method [272]. Therefore, stressed and damaged organisms will always give lower recoveries than intact organisms.

As such, when looking between all the papers included in this review, target organism and strain is just one of the many factors that can be attributed to the wide range of recoveries seen in this review in addition to showing the difficulty when trying to view results comparatively. All papers (N= 73) report these wide range of recoveries, an example of this is a range between, 0.7%-52.2%, depending on surface

type and sampling method chosen [167] All the potential factors causing variation in recoveries is explored in table 2.4.

2.4.2.2 **Processing**

Different methods and additional steps for processing and options to improve recovery are available. These are discussed below;

2.4.2.3 **Wetting agents**

Swabs, sponges and wipe methods can be enhanced by pre-wetting before surface sampling. There are many options for wetting agent, ranging from sterile saline [169] buffered peptone water, various strengths of Ringer solution and Letheen broth [263].

Phosphate-buffered saline was best for *E. coli* and *B. cereus*, whereas phosphate-buffered saline with tween was better for *Burkholderia thailandensis* when compared with Butterfields buffer and maximum recovery diluent (MRD). However, one of the buffers tested, Butterfield's buffer, had a marked reduction if used with *E. coli*, from 60.6% to just 40.5% [283]. All swabs were significantly improved by pre-moistening [168] [263]. A dry cotton swab gives 8.0% recovery and by pre-moistening, improves to 41.7% [168]. This is further supported by other work [169] where all swabs were improved by pre-moistening, improving from 57.5% positive rate dry, to 83.4% positive moistened [169]. Cotton tipped swabs in ¼ strength ringers solution was best for *E. coli* [263]. Cyto-brush textured swabs in COPAN rinse formula was best for *S. aureus* [263]. Wetting solutions with Letheen broth and solutions with buffered peptone water significantly increased bacterial numbers of *S. aureus* and *E. coli* by 6.5 log at room temperature. Storage in maximum recovery

diluent (MRD) increased *E. coli* and *S. aureus* recovery, though to no statistical significance. As some wetting agents can increase bacteria recovery, and are designed as such, like as MRD, these are not appropriate for total counts, and should be used as presence-absence assessment only as introduction of such wetting agents will confound results and lead to higher counts.

2.4.2.4 **TRANSPORT MEDIUM AND CONDITIONS**

Transport medium is a solution for sample storage during transport to the lab for processing. This medium minimises or supports growth within the sample and can be made from a number of mediums such as; anaerobic universal transport medium, aerobic Amies medium [287] or neutralising buffer [170]. Choice of transport medium is important, [287] and the choice should vary between the target organism, time taken to transport to the lab, and post-test storage conditions and storage time, as some transport mediums allow inhibition of growth in the sample when being stored at room temperature, yet this would not be appropriate for samples that could be refrigerated immediately [287]. Unsurprisingly, storage time has an impact on percentage recovery [287]. Polyurethane swabs without transport medium gave best recovery for *Clostridium innocuum, Fusobacterium neocrophorum, Clostridium perfringens, P. tetradius, Peptostreptococcus anaerobius, E. coli, S. aureus, Streptococcus pneumoniae, Neisseria gonorrhoea, Haemophilus influenzae* [287]. An exception to this was *Bacteroides fragilis*, where best recovery was achieved with polyurethane swabs without transport medium. Addition of transport medium caused decrease in percentage recovery for all swabs for all bacteria tested [287]. This is explained due to the reduction of dilution factor by adding a transport agent

and due to less adhesion of the organism to the dry swab material, allowing easier release [287].

2.4.2.5 SAMPLE EXTRACTION

Swab and sponge samples require extraction in order to undergo further processing. Ensuring optimum extraction of the sample is important to reduce these associated losses. Vortexing, agitation or sonication of the swab or sponge are three methods that allow removal of the sample from the sampling device. An optimum time of two minutes vortexing was shown to be superior over 12 minutes of sonication followed by agitation to remove *B. anthracis* spores from a swab [168].

There are many types of extraction solutions; phosphate-buffered saline, Butterfields's buffer, Butterfield's buffer and tween, MRD [283]. After target organism, choice of extraction solution was found to have the next biggest impact on extraction efficiency [283].

Most losses occur during processing, such as vortexing, [178] and variation in counts occur when incorrect swabbing medium is selected, or the samples are improperly stored, such as keeping the samples at ambient temperature or failing to process them quickly [263]. Organisms can attach differently depending on the swab material, the charge of the swab and the organism, and the wetting agent used. In addition, different swab materials have varying pore sizes from polyurethane with smaller pore sizes and cellulose sponges with irregular pore shapes and sizes [288].

Highest recoveries were found, for both adsorbed and unadsorbed cells, with contact plate and dipslides against swabs, as these direct contact methods negate the processing losses that are caused by recovering the sample from the swab [269].

Vortexing improved recovery of flocked swabs from 60 to 76%. Rayon swabs were not improved by vortexing [275]. Overall, vortexing gave the best results, except for polyester swabs, which gave better results with sonication [168]. Highlighting the importance of processing, depending on pre-moistening and the use of vortexing, recovery with swabs can vary between <0.01-43.6% [168].

2.4.2.6 **Enrichment**

Enrichment is the process of placing the sample into a broth and incubating. This allows organisms time to grow in favourable conditions, and can be useful for slower growing organisms, or cells that have become stressed from environmental pressures or damage from sampling. Enrichment can be non-specific, to support growth generally of all organisms within a culture (nutrient is commonly used) or specific, replicating the required conditions that selects for certain organisms, such as mannitol salt broth, which has a high concentration of salt (7.5-10%) which inhibits most Gram-negative organisms, while promoting the growth of *Staphylococcus* spp [289].

Following growth, aliquots are then subcultured from this broth and plated out onto various selective or non-selective media. A commonly used broth is brain-heart infusion broth. N=16 studies sampling the hospital environment used subculturing for their samples. Broth composition and incubation time and temperature will vary on organism of interest. One study found that enrichment in Tryptone soy broth improves detection rate from 61.3% to 80% for *S. aureus* [169]. However, this produces a presence-absence result, not a quantifiable CFU.

2.4.3 **Incubation**

Incubation times and temperatures vary between the literature, yet it is shown that time and temperature have an impact on the final results from sampling. Choice of incubation temperature can have an impact on growth or recovery of an organism, such as thermophobic or thermophilic strains. When exposed to environmental stressors, optimal temperature becomes more important. A study by Paksanont *et al.* 2018 found that *Burkholderia pseudomallei* (a Gram-negative motile rod) had the strongest tolerance to environmental stressors (salt stress, hydrogen peroxide exposure) at 37°C when compared with a range of temperatures between 25-42°C [290]. Within industry, the temperature window is usually ±5°C. As demonstrated by Pakasnont *et al.* 2018 a 2°C growth temperature can impact % recovery of an organism under stress [290], though more data is needed to assess clinically-significant environmental pathogens under different stressors to determine how important this incubation window is for environmental surface samples .

N=11 studies incubated at 37°C 24-48hrs and N=7 incubated at 35°C for 24-48hrs.

2.4.4 **Sampling bias**

When trying to make conclusions and comparisons between the literature, it is important to consider a wide range of potential sampling bias. Firstly, sampling sites and number of samples taken varies considerable between each report. Number of samples taken range between 24-2532, [209] [219] giving an average (mean) of 464 samples across all studies included in this review. Percentage of surfaces reporting contamination will vary depending on surfaces chosen for each

experiment, in combination with target organism. Certain combinations of target surface and organism will likely give positive results, such as looking for coagulase-negative *Staphylococci* (CoNS) on patient charts, which will be handled by personnel without gloves, which gave up to 100% contamination [218] [282]. In contrast, looking for Gram-negative organisms, which are found significantly less in the hospital environment than Gram-positives [219] will undoubtedly be reflected in lower recoveries. While it has been reported that Gram-negatives are found less within the clinical environment, it is important to consider that Gram-negative organisms are more susceptible to cleaning agents due to their thin cell wall when compared with Gram-positives, which have a thick protective peptidoglycan wall, which is harder for cleaning agents to penetrate [291]. In Gram-positive organisms, peptidoglycan cell wall makes up 90% of the dry cell wall weight, compared with Gram-negative organisms, in which the dry weight of the cell wall is just 10% [291, 292]. It is this lack of thicker peptidoglycan layer that mean Gram-negatives are more susceptible to desiccation compared with Gram-positives [291]. Gram-positive organisms, such as *S. aureus*, are known for their strong tolerance against desiccation [285] and has proven survival on plastic surfaces for >1,000 days [284]. Therefore, there may not be specifically fewer Gram-negatives in the surface environment compared with Gram-positive organisms, it is just that they are cleaned more effectively due to their structure and cannot persist as long due to desiccation [286, 293]. Also, the study by Lemmen *et al.* 2004 used traditional surface sampling devices, swabs and contact plates, which have been shown to recover, on average (mean), fewer Gram-negatives than Gram-positives (Gram-positive *S. aureus* and *E.*

faecalis recoveries were 24.27% and 23.08% compared with the Gram-negative organisms *K. pneumoniae* and *P. aeruginosa* which gave 20.01% and 11.57% respectively) when tested directly on a range of surface materials with a range of samplers (see sections 3.4.2- 3.5.2). It is proposed that while results show in some settings, Gram-negative organisms may survive better in the clinical surface environment [219] this is not the case worldwide; in an overcrowded tertiary hospital in India, Taneja *et al.* (2005) reported of 332 contaminated environmental samples 61.1% were contaminated with Gram-negative bacteria, and 65% with Gram-positive cocci, with the highest concentrations of contamination found in the Neonatal ICU [99]. Pediatric ICU's have been linked to other Gram-negative outbreaks [42] and this study suggests the MDR *K. pneumoniae* source was environmental, from a contaminated fridge that stored blood bags, facilitated by healthcare workers' hands, though this link was an assumed most-likely observation as typing studies were not undertaken [99].

Healthcare setting and hospital specialty can play a role, and it is important to consider results in light of where they have been recovered from. Rooms specifically for patients colonised with certain organisms, such as MRSA, will allow enhanced shedding into the environment and therefore sampling will recover more of these organisms than in other areas of the hospital. In addition, some studies sample rooms previously occupied by infected or colonised patients, in order to assess risk to the next patient. These rooms have been shown to carry contamination from the prior occupant, posing a risk of cross-contamination to the next patient. This will give a difference in sampling recoveries and organisms that are found, and

such studies cannot be used as an example for the whole hospital environment. Also, some studies concentrate on the high-touch objects and near-patient environment, whereas other studies sample the wider hospital environment and shared communal spaces, which will give different results.

As previously explored, choice of sampling method will differ between each study, which will have an impact on recoveries as sometimes sub-optimal methods may have been used.

2.4.5 **SURFACE STATE**

Surface state is another influential factor that causes variance in method efficacies, as depicted in table 2.3. Frequently reported in the literature is the effect of recovery when the cells are dried or adsorbed to a surface. Sampling from a wet or dry surface has a great impact in individual method ability to recover cells from a surface. Dry surfaces always have lower recovery [177]. It is easier to recover from a wet surface than a dry surface [177]. Dry surface represent the bulk of the hospital environment, though bathroom, sluice, and sink-adjacent surfaces may be wet. Better recoveries are achieved for *L. monocytogenes* on wet surfaces compared to dry [294]. Brush textured swabs also had poorer recoveries on dry surfaces [263]. However, this was contradicted where *S. aureus* had significantly better recovery from the dry surface than wet, 10-65% against 40-77% dry [263]. While additional assessment is needed to judge the significance of the losses from wet or dry surfaces, Moore *et al.* 2001 found that for self-contained qualitative and semi-quantitative swabs (coliform SwabCheck, Pat-Chel and Coli Trace) on dry surfaces, the lack of

diluent and extraction step, for dry surfaces, increases recovery tenfold [177]. This implies extraction is a more critical limiting factor than surface state [177].

Drying of a virus on a surface significantly reduces recovery for macrofoam swab. A 24hr drying time gave 18.2%-25.7% recovery whereas 48hr drying reduces this recovery to just 10.0% [276]. Significant differences are caused between sampling efficiencies between adsorbed and unadsorbed cells [269].

Surface bioburden is an important consideration [281] for highly contaminated surfaces, sponges were significantly better for recovering *C. difficile* ($P < 0.05$) than contact plates. Sponges can detect *C. difficile* at <10 CFU spores, and gave recoveries of 94.4% on poly work surfaces, 94.4% stainless steel, 83.3% bed rail. Contact plates had no recovery on all surfaces at same inoculation concentration [281]. However, these spore-forming organisms such as *C. difficile*, may or may not be directly extrapolated to vegetative organisms. Levels of surface contamination play a role; macrofoam swabs were more sensitive (1.0×10^{0}-3.9×10^{-1}) than contact plates (on mattress surface only, at 2.4×10^{0}) and other swabs (except for the neutralising swab on a bench surface, which was 2.6×10^{1}), as they can give positive results at the lowest levels of MRSA concentration (1.0×10^{0}) [170]. Swabs gave the best recovery at higher loads, whereas contact plates were better for lower surface loading. At 5×10^{6} CFU/ml swabs could not detect *Klebsiella pneumoniae* on a dry cotton surface, while at the same concentration (5×10^{6} CFU/ml) contact plates could detect *K. pneumoniae* [268].

Surface size is an important consideration when choosing a sampling method. Contact plates and dipslides are limited, whereas swabs can sample a larger surface area with ease, and sponges a bigger area still. When sampling larger surfaces, it was found macrofoam swabs had the best percentage recovery (43.5%) of norovirus from a large surface [276].

Surface material causes variance in recovery [170]. It causes significant variance in recovery of *L. monocytogenes* [294]. Surface material gives differences in recoveries regardless of sampling methodology. The best recoveries were found on glass surfaces, and the worst recovery on finished concrete [167]. It was easier to recover *B. subtilis* from a smooth surface like vinyl than carpet [178]. Contact plate and swab combinations gave best results on stainless steel surfaces [275]. Surface material causes significant difference on MRSA recovery [270] [281] [273]. The impact of surface material and effect on surface sampling devices is tested and further explored in chapter 3.

Another important state of surfaces is residual chemicals, which may or may not interfere with recoveries of organisms. One study in the review considered this, and it was found that residual disinfectants do not interfere with *S. aureus* colony counts on contact plates, [295] however further studies on a range of surfaces with different organisms and recovery methods should be undertaken before a conclusion can be reached. Hospital surfaces are cleaned with a variety of products with different active ingredients (discussed in Chapter 4, with cleaning agents specific to this thesis in table 5.1). Some cleaning agents do not leave residues on surfaces, such as alcohol. Other cleaning agents, such as bleach-based cleaners, can leave residues

which can impact sampling. In relation to cleaning agents used within different spaces, neutralising agents should be carefully selected [260]. The ward-sampling study allowed a 3-hour post-cleaning window to allow chlorine residues to dissipate before taking samples (section 6.3.4). In addition to this, the contact plates contained the neutraliser sodium thiosulfate, which can further deactivate chlorine. This works by thiosulfate reacting with the hypochlorite, which is the active ingredient in bleach-based cleaners, and oxidizing it to sulfate [296].

2.5 GAP ANALYSIS

The review of the literature revealed inconsistencies in methodologies and testing different organisms under different conditions that would have an effect on recovery. Table 2.4 below makes overall conclusions on sampling under different conditions based on the data of all available organisms. However, much of this data is based on pathogens that are of less significance in terms of the clinical surface environment, such as food-bourne pathogens like *L. monocytogenes.* To highlight the lack of data when considering important clinical pathogens, the available literature can be assessed for data concerning the recovery of 5 organisms of great clinical importance, tested under the various conditions that have been proven to cause wide variances in recoveries.

Table 2.4 Wide gaps in literature as demonstrated by number of publications testing five clinically significant pathogens of concern under different conditions.

(*n/a = no data available for these organisms)

	S. aureus	*K. pneumoniae*	*E. faecalis*	*P. aeruginosa*	*C. difficile*
Metal surfaces	[269, 270, 273, 297]	*n/a Addressed in Chapter 3	*n/a Addressed in Section 2.4	[270]	[281]
Fabrics	[268]	*n/a	*n/a	[268]	*n/a
Plastic surfaces	[270]	*n/a Addressed in Section 2.4	*n/a Addressed in Section 2.4	*n/a Addressed in Section 2.4	[281]
Glass surfaces	[273]	*n/a	*n/a	*n/a	*n/a
General porous surface	*n/a	*n/a	*n/a	*n/a	*n/a
General non-porous surfaces	[271]	*n/a Addressed in Section 2.4	*n/a Addressed in Section 2.4	*n/a Addressed in Section 2.4	*n/a
Wet or dry surfaces	[263]	*n/a	*n/a	*n/a	*n/a
Surface size	*n/a	*n/a	*n/a	*n/a	*n/a
High or low bioburden	[170, 267]	*n/a	*n/a	*n/a	[281]
Adsorbed or dried cells	[269]	*n/a	*n/a	*n/a	*n/a
Stressed or injured cells	*n/a	*n/a	*n/a	*n/a	*n/a
Cleaning agent residue	[295]	*n/a	*n/a	*n/a	*n/a
Presence of soiling	[273]	*n/a	*n/a	*n/a	*n/a
Uneven surfaces	*n/a	*n/a	*n/a	*n/a	*n/a
Sampling device brand	[267]	*n/a	*n/a	*n/a	*n/a
Wetting solution type	[263, 287]	*n/a	*n/a	*n/a	*n/a
Transport and storage conditions	[287]	*n/a	*n/a	*n/a	*n/a

Here, the lack of available data is clear by the cells without citation. With these large gaps in the literature, it is difficult to know just how well surface sampling devices will perform when used in the real clinical environment, as many of these factors will be critical. Additionally, several of these factors will likely be occurring at the same time due to the unpredictable nature of the clinical environment and heavy traffic of people and patients. For example, on a clinical surface, you might expect cleaning agent residue as surfaces are regularly cleaned, presence of soiling if the surface is near a patient or in the sluice area, stressed or injured cells due to exposure to the harsh environment, with a varying amount of surface types. Some surfaces are also made of multiple materials, such as chairs and over-bed tables. Importantly, when assessing the pathogens that do have available data for different testing conditions, the number of studies is a concerning factor. Some factors are considered only in single studies. *S. aureus* had the most sampling factors tested and, unlike the other pathogens, had more than a single study for some factors.

2.6 SETTING UP AN ENVIRONMENTAL MONITORING PROGRAMME. WHAT CAN WE LEARN FROM INDUSTRY GUIDELINES?

Within the food and pharmaceutical industry, there are comprehensive, strict guidelines available for environmental monitoring, as well as guidance on how to set up, implement, and maintain environment monitoring programmes for different types of environments, such as sterile or non-sterile processing environments. The guidelines and governing bodies followed depends on both geographical location, industry type and environment. The following guidelines were considered to explore the key components that constitute an effective environmental monitoring programme; the EU 'Orange Guide', US Food and Drug Administration (FDA), United States Pharmacopeia (USP) and International Organisation for Standardisation (ISO).

The EU 'Orange Guide' is a critical set of guidelines used in European and UK pharmaceutical laboratories. This guide provides a single reference material for Europe relating to all matters of the industry related to human medicine production, ensuring lab practise is both safe and within the law. For surface sampling, Annex 1, guidance on good manufacturing practise, manufacture of sterile products, can be referenced. In this document, it highlights when and how to use contact plates, settle plates, swabs, finger dabs and air sampling devices to monitor cleanrooms. It also gives guidance on how much contamination is allowed before failure occurs, in different grades of cleanrooms (classified as A-D), which require different levels of clean.

Table 2.5 Produced from recommended limits for monitoring clean areas during operation. EU Guidelines to Good Manufacturing Practise Medicinal Products for Human and Vetinary Use. Annex 1.

Grade	Contact Plates CFU/plate	Gloves print CFU/ glove
A	<1	<1
B	5	5
C	25	-
D	50	-

The United States Pharmacopeia, USP, is a set of guidance legally recognised in the U.S and more than 140 other countries. This guidance is similar to the Orange Guide, in providing standards relating to all components related to producing drugs and regulating the environments during human and animal drug manufacture. For surface sampling (USP 1116) Microbiological Control and Monitoring of Aseptic Processing Environments, can provide some insight into the guidelines associated with surface sampling of cleanrooms and controlled environments. This document contains suggested limits and information on how to undertake investigations if these limits are exceeded. There is also comprehensive guidance on how to set up a good environmental monitoring programme. Here, it is noted that all components of sampling need to be controlled, including; culture media used, quantification method, incubation times and temperatures. USP chapter 1113 also has further information on processing samples; microbial characterisation, Identification, and straintTyping, workflow for microbial identification.

Table 2.6 Suggested initial contamination recovery rate (rate at which any contamination is found, the incidence) in aseptic environments, adapted from Table 3 USP <1116>

Room Classification	Contact plate/ swab (%)	Glove/ garment (%)
Isolator/ISO 5+	<0.1	<0.1
ISO 5	<1	<1
ISO 6	<3	<3
ISO 7	<5	<5
ISO 8	<10	<10

The US Food and Drug administration (FDA) have details on acceptable environmental monitoring methods within their aseptic processing guidance document. This document considers all the important components involved with sterile manufacture. The components within this document are not legally mandated, and are suggestive, though adherence to its contents will ensure the applicable statues and regulations are satisfied. It gives guidelines on where to sample, such as product contact surfaces, floors, walls and other equipment. There is also guidance on wider environmental monitoring. Other factors such as technician competency and training is also covered here. Recommended microbiological action limits for clean rooms were omitted here, as they provided only active and passive air sampling limits, and allowed particle sizes, which is not applicable to surface sampling.

From these industry guidelines, components can be identified that must form part of an environmental monitoring programme. However, it is important to

consider how findings from the food and pharmaceutical industries cannot be directly extrapolated to clinical settings for several reasons. Clinical settings are not controlled, and the source of contamination (patients) is variable and cannot be controlled. Additionally, the goal of the clinical environment is not to produce a sterile environment, which is not feasible, only to reduce pathogen load by cleaning. Finally, unlike in the pharmaceutical environment, there is no specific defined end of activity in which sampling and cleaning can occur. With these limitations in mind, the following components that should be contained in the programme were identified:

- Where to sample, with sampling map
- How often to sample
- What to sample with, with consideration of;
- The type of result required (qualitative/ quantitative)
- Sampling technique
- Training and competency of sampling technicians (reference to local training and competency testing)
- If samples are not processed in house; guidelines on appropriate storage and how to send samples, and required timelines of each
- How to process samples; media choices, incubation times and temperature, storage allowances (i.e samples must be refrigerated and processed within 6 hours)
- Action and Warning limits/ pass or fail criteria
- Process of launching an investigation upon failure and actions to be taken
- Details of investigation procedure; who to notify with contact information, format of report, information that must be included
- Follow up of investigations and how long such investigations should take
- How to analyse results and report these results
- Environment trending
- How results are stored (reference to local Quality Management Systems)

Figure 2.7 Components for an effective environmental monitoring programme

Setting pass and fail criteria is, perhaps, the most challenging component of designing the environmental monitoring programme. These limits are well documented for industry, as shown in tables 2.5, 2.6, and 2.7. These limits are carefully broken down by how critical each area is, with varying levels of CFU allowed before failure. Currently, the only available guidance for clinical surfaces are arbitrary guidelines as set out by Griffith and Dancer, suggesting limits of 2.5CFU/cm^2 per plate and 5CFU/cm^2 per plate. While these guidelines make a critical step to suggest limits, they are not evidenced-based in relation to impact and specific patient risk and reduction of HCAI, and further work needs to be undertaken.

While routine environmental monitoring of clinical spaces is not mandated and there is a limit on the level of sampling that could be routinely taken within the clinical space in regards to resourcing limitations, the questions should perhaps not be posed in terms of what could be gained with routine sampling, but what the consequences may be by choosing not to monitor the environment. Sampling following outbreak is a ‘catch up’ exercise in response to patients presenting with clinical infection or colonisation of an organism. It takes time to identify trends in infection rates to be attributed as an outbreak scenario rather than coincidence, which means that at the time of starting investigation, several patients have become infected leading to at best increased bed days, or worse outcomes including use of antimicrobials which is already a concerning issue (section 1.3.7) or death. A study by Hong *et al.* 2012 in a PICU began investigation following 3 clustered cases of fatal imipenem-resistant *A. baumannii.* A total of 36 patients were reported as positive between 2001-2011. Of which, 20 patients were reported as infected or colonised

between 2010-2011 [98]. Environmental sampling to find the source of the outbreak began, and the PICU sink was revealed as the source of the outbreak for the cluster [98]. The value of routine monitoring is clear in this case. If routine monitoring had been undertaken within the environment, the outbreak source could have been identified earlier, and cleaning interventions could have stopped the outbreak before more patients became infected. Outbreak events are multifaceted, and it cannot be determined if it were just the sink alone that caused the outbreak, and how many cases if any prior to the cluster the sink were responsible for, but this sink could provide an important sentinel site for this particular environment. Going ahead, this sink and other sinks within that PICU could be monitored routinely to inform environmental trending results so reservoirs of infection, in this instance *A. baumannii*, could be identified before an outbreak occurred, or at least given faster reaction times of implementing response strategies such as enhanced cleaning as patients began to present with clinical infection. Data is needed to identify just how much of an impact routine monitoring might have in such scenarios, as evidence-based results showing environmental monitoring can proactively prevent outbreak would be data to leverage in suggesting large scale roll-out of such monitoring programmes and giving the required cost-benefit analysis of such programmes. Within the NHS, due to limited budgets, any IPC strategy considered for implementation must have supporting evidence that the impact of implementing the strategy will far outweigh the cost.

Following the assessment of what constitutes as a complete environmental monitoring programme and surface sampling recommendations, the following

guideline was created, in the style of a flow chart, to allow a technician to make evidence-based choices on their use of sampling devices depending on their individual needs and type of environment they have chosen to sample.

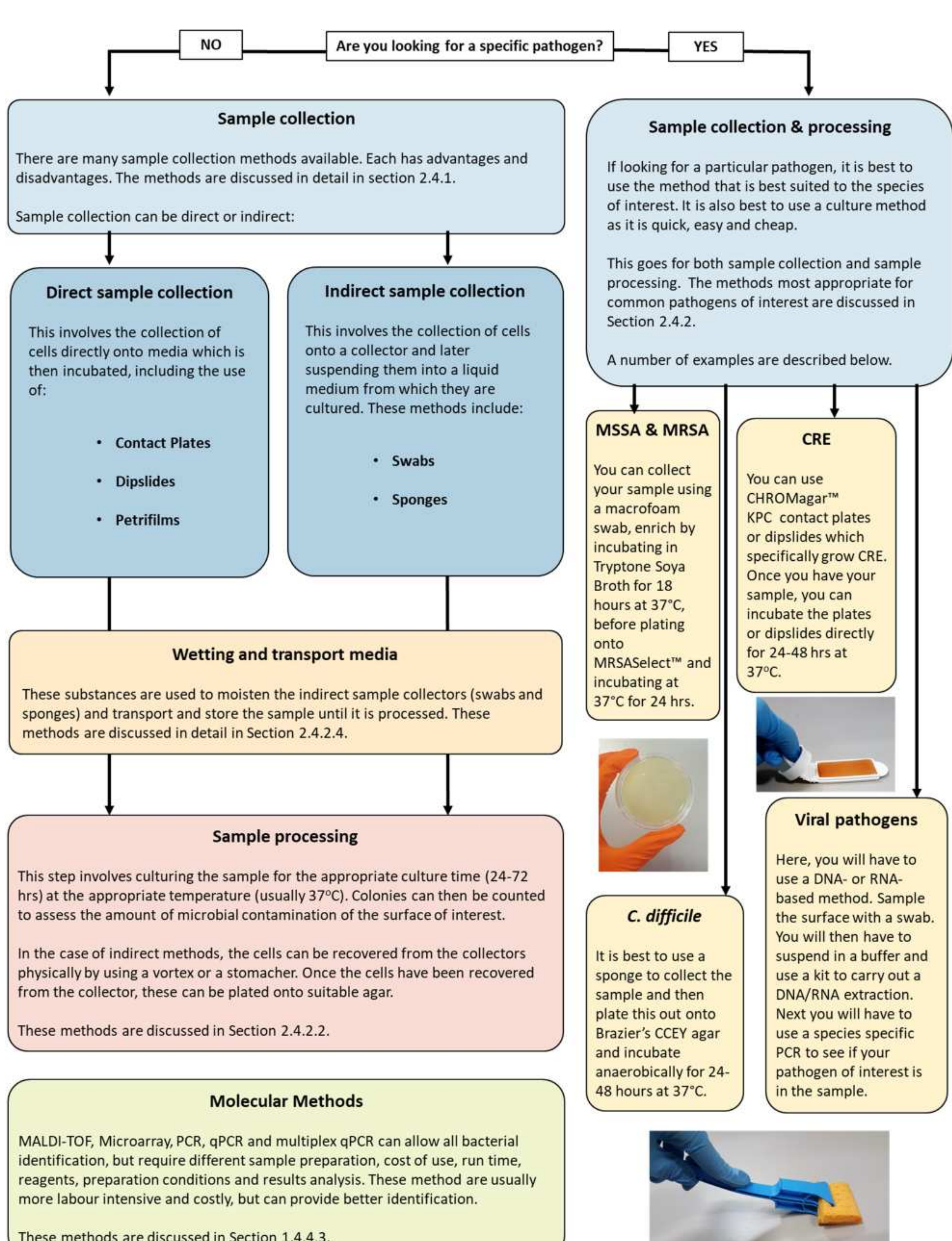


Figure 2.8 Flow diagram outlining findings from the review while making suggestions depending on individual sampling needs based on (Rawlinson, Ciric et al. 2019).

2.7 CONCLUSION

The lack of guidelines for sampling the clinical environment, how to set up and maintain an environmental monitoring programme, and the discrepancies between surface sampling devices make the task of environmental monitoring confusing and difficult. As routine surface sampling is not currently mandated for the clinical environment outside of outbreak scenario, many hospitals simply choose not to monitor their environments outside of outbreak scenarios. Surface sampling devices should be carefully chosen depending on the research question, if the assessment is specific pathogen identification, which is important and useful during outbreak of a specific pathogen, in which selective sampling techniques should be used (contact plates or dipslides with selective agar) for quantitative assessment. If qualitative assessment is needed, then enrichment methods could be used to increase sensitivity. If the assessment is general cleanliness and a quantitative result is needed, then direct contact methods with general agar and a neutraliser (TSA) is a good selection. For a faster, semi-quantitative measurement of cleaning, ATP-based swab sampling could be used. However, availability of such methods is limiting, so trust may choose to utilize sampling devices already readily available within their environment, such as swabs, and maximise on efficacy, by adding a wetting solution (such as Letheen broth for S. aureus or E. coli) and stored in MRD to increase recovery then vortexing for no less than 60 seconds. Choice of method will restrict the type of result produced, so research question should be assessed prior to selecting a sampling device.

While there are several circumstances which would leave a trust choosing not to sample, such as lack of guidance, resources, time, or skilled staff, the review attempted to offer useful suggestions to circumvent these issues, such as cheaper sampling alternatives, sampling devices that could take faster samples, or choices which do not require a microbiologist to analyse and interpret, such as ATP or UV-marker based methods, or total plate counts.

Finding contradictory or difficult to read literature could be the difference between a trust choosing not to sample their surfaces, or making poor sampling choices producing skewed data. Here, this work attempted to provide an easy to read and accessible review of all the options, with real suggestions that could be implemented within the clinical environment, allowing technicians to make informed decisions about their surface sampling. As more work needs to be done to provide a complete picture of the surface sampling options and how they may perform under different testing conditions, particularly in relation to clinically-significant pathogens and those that are the focus within this thesis (*S. aureus, K. pneumoniae, E. faecalis, P. aeruginosa*) these should be tested on a range of surfaces relevant to the clinical space. Gaps were identified from the review (table 2.4) and chapter 3 completes this data further.

Chapter 3 EFFICACY TESTING OF SURFACE SAMPLING DEVICES

3.1 INTRODUCTION

Many multi-drug resistant organisms have been recovered from hospital surfaces [139]. Transmission of pathogens between the patient, surface environment, and further spread in the ward environment has been documented [208]. It has been established that surfaces and the environment play an important role in the transmission of healthcare associated infections (HCAIs), and breaking the assumptions of surfaces being 'low risk' is an important goal to ensure hospitals are, overall, a cleaner and safer environment.

Knowing where the contamination is in the environment, what organisms make up each specific clinical environment and identification of reservoirs is critical. As such, development of an effective environmental monitoring programme is important. Monitoring the clinical surface environment can be in the form of specific pathogen detection, or general sampling to monitor cleaning efficacy [170]. However, routine sampling of the clinical surface environment is not mandated. This lack of guidance has further difficulty in the lack of comprehensive information on surface sampling devices. The clinical environment consists of different surfaces, pathogens, organic matter and cleaning agent residues, all of which will affect the efficacy of a sampling device. Therefore, knowing how to sample these surfaces can be difficult. A large gap in the literature has only fueled the difficulty in knowing how to properly sample surfaces.

The clinical surface environment is made of many different types of surfaces, under different conditions. Real clinical surfaces are subjected to a variety of factors

that may cause a drastic change in the efficacy of different surface sampling devices. Due to the ever-changing and fast-paced nature of hospital environments, it is not possible to control these factors, but to consider their impact on different sampling devices and to make an informed choice for different types of surfaces and environments. Factors such as different target organism or strain [272, 283], surface type [168, 268, 270, 280] or surface state [177], have all been shown to cause variability in recoveries (section 2.4.2.1). As such, it is critical to prevent inappropriate selection of sampling devices which could skew results, either by poor or no recovery, or overestimation of a certain species or strain.

The surface environment of the hospital is made of a range of surface types and materials. Some surfaces are unsuitable for certain sampling devices, such as using contact plates for uneven surfaces [139, 268] or wet surfaces, in which they perform poorly. As the clinical environment is made of many different surface types and textures, these impacts are of concern when choosing a sampling device. Knowing how surfaces play a role in organism recovery is vital to make an informed choice when selecting sampling devices. Difficulty in recovering organisms from certain surfaces could be due to the ability of organisms to adhere and form biofilm on some surfaces more easily than others. Surface charge also plays a role in bacterial attraction, as negatively charged surfaces have been found to attract more bacteria than positively charged surfaces [298].

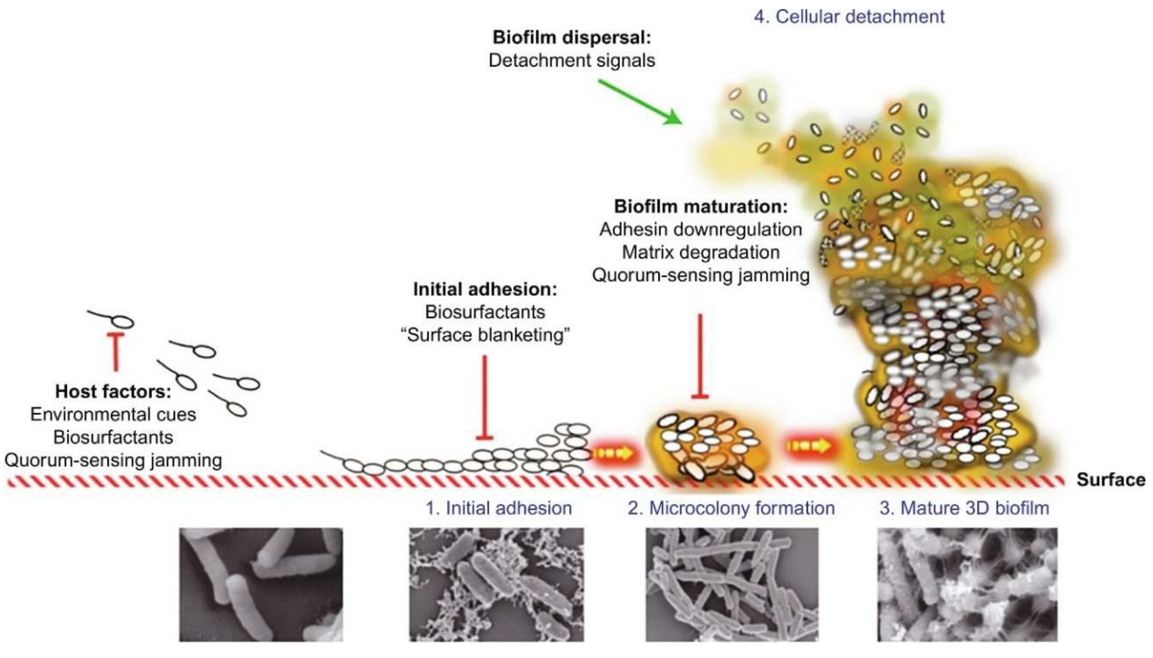


Figure 3.1 The process of biofilm formation on a surface as taken from Hadla 2018 [299].

Organisms can attach and adhere to surfaces, and persist in a biofilm [300, 301]. This occurs in most environments [301] Biofilms protect organisms from desiccation, surface cleaning and abrasion [300]. Organisms in a biofilm are also more resistant to antibiotics and cleaning agents. Figure 3.1 depicts the process of biofilm formation on a surface. External or internal factors send signals to organisms to form biofilm, starting the initial adhesion stage, which takes approximately 1 minute involving hydrodynamic and electrostatic interactions [302]. Cells weakly and reversibly attach to the surface using Van der Waals forces [152, 303]. The next stage is permanent or irreversible attachment, where cells utilize their structure in order to adhere to the surface, using extracellular polymer substances (EPS) components and pili to anchor themselves (Figure 3.2A) [152, 303].

EPS are produced by a wide range of organisms and are polymers consisting mostly of polysaccharides, proteins and DNA. *Pseudomonas* spp. and species closely related to the *Pseudomonas* genus are known to readily produce EPS [304]. EPS play a role in nutrient entrapment and protection from environmental stressors and are produced when triggered by environmental signals [305]. EPS often have long chains and are most frequently negatively charged, though neutral and positively charged EPS do exist. EPS provides good adhesion to polar surfaces [305]. Pili (shown in figure 3.2) are long flexible fibers assembled in the bacterial envelope and aid surface attachment and motility, and increase the initial rate of attachment and the rate of conversion to irreversible attachment [306]. A key cleaning moment is intervention prior to this irreversible attachment. Cleaning at this stage will allow easier and more complete removal of organisms, as the cells have not strongly attached yet to a surface. Organisms can have different types of pili which aid binding to surfaces. Gram-negative and Gram-positive organisms can both have pili, though they are far more diverse in Gram-negatives [306]. This is a factor that allows Gram-negatives, such as *P. aeruginosa,* to form such strong biofilm and resist cleaning. The irreversible attachment process takes several hours and involves van der Waals interactions between the cells walls and the surface (Figure 3,2B) [302].

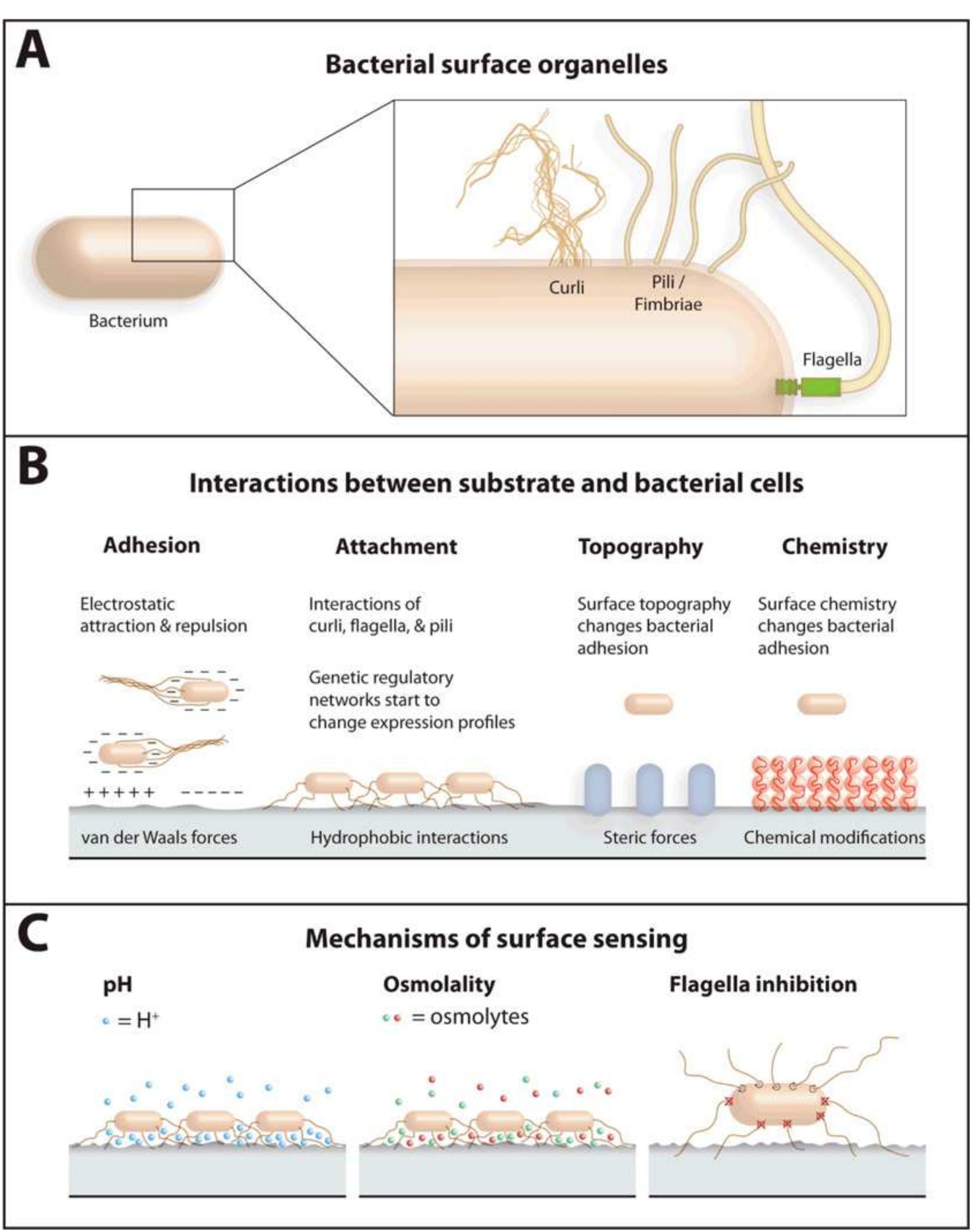


Figure 3.2 Bacteria-surface interactions. (A) bacterial proporties used to aid attachment to a surface, including curli, pili and flagella. (B) surface proporty factors that bacteria use to attach (or be repelled) by surfaces (C) how proximity to a surface causes a change in how the bacteria expresses certain genes. Taken from Tuson 2013 [302].

Exposure to the environment can cause a phenotypical change [300], allowing an organism to express certain genes that allow biofilm formation. This varies between different organisms, however. *Staphylococcus aureus* has 3 genes for reversible attachment, irreversible attachment, microcolony attachment, mature biofilm and dispersal. In contrast, *P. aeruginosa* has 12 [307]. Once permanent attachment has formed, by way of biofilm, removal from a surface is difficult and requires robust cleaning using physical force and detergents to break down the biofilm before disinfection can occur.

Organisms adhered to surfaces are more difficult to recover with sampling devices than non-adhered cells [308]. Surface texture will help, inhibit, or assist in bacterial adhesion; even microscopic adhesions or scratches on the material surface will produce an excellent surface for bacterial attachment. For example, steel surfaces can have flaws that can harbor cells [309]. Even individual finish on the same material can have an impact on how easily cells can attach; a study by Arnold and Bailey (2000) found that the finishing treatments between stainless steel had an impact on bacterial contamination [110]. They tested raw and untreated, sandblasted, sanded and electropolished steel coupons. Early biofilm formation can be slowed by mechanical or electrochemical treatments of the stainless steel, and that decreases in the roughness of the surface reduced bacterial contamination, and early biofilm as shown by scanning electron microscopy (SEM) [110]. The electropolished steel had the fewest attached organisms under SEM, and also had the lowest mean roughness values [110].

Smooth (free of visible abrasions), new or undamaged surfaces do not allow such easy attachment. It has also been identified that surface topography (shape of the surface) plays a role in attachment, and sizes close to organism size may allow entrapment and can occur at 0.9µm [298] which would make recovery more difficult. As all organisms are different shapes and sizes, this entrapment will vary.

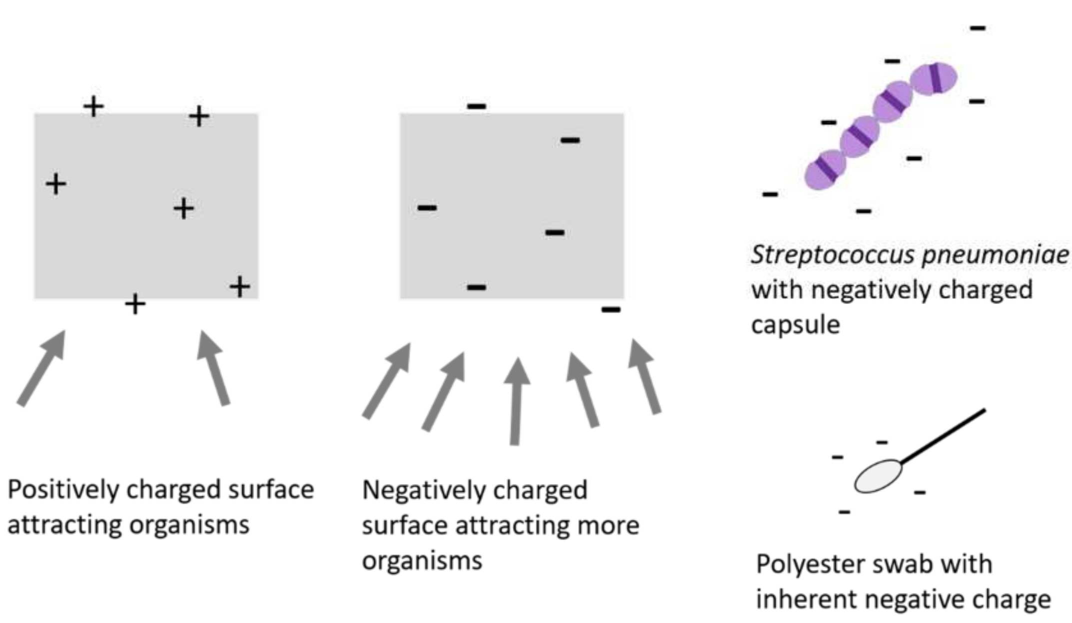


Figure 3.3 Interactions of positive and negative charges on surfaces, organisms, and surface sampling devices

However, the reality is more complex, when there is the additional factor of how different organisms are recovered better from some surfaces. Different surfaces have different surface charges [310, 311]. The surface energy of plastic and metal have been measured as 42 and 850 dynes respectively [311]. In relation to cleaning and environmental contamination, surface change also plays a role in how Gram-positive and Gram-negative organisms adhere to surfaces. A study by Gottenbos *et al.* (2001) found that both Gram-negatives and Gram-positives adhered most rapidly

to the positively charged plastic surfaces, though there was no subsequent growth of the Gram-negative strains, suggesting bacterial adhesion is faster on positively charged surfaces, that positively charged surfaces attract more bacteria, and that a negatively charged surface may reduce the chances of bacterial adhesion [312]. As this adhesion is a critical step in biofilm formation, this should be considered when designing hospital surfaces.

Like surfaces, different organisms have been shown to have a variety of surface charge depending on the species [313] and most bacteria carry a net negative surface charge [314, 315]. It is feasible to suggest that interactions between the charges of surfaces and organisms could result in weak or strong attachment and attraction to a surface, and therefore have some impact on recovery with a surface sampling device. The impact of these charges, however, may or may not be mitigated by the use of appropriate pressure when using a sampling device, or selecting a swab sampler which allows exertion of a great deal of friction when sampling. Additionally, different swab materials have different charges, shown in figure 3.2; swabs can produce a charge during use due to friction, while cotton swabs are neutral and the fibres could produce a negative charge, aiding recovery [311]. Whereas synthetic fibers such as flocked nylon or polyester swabs do not have a charge [263]. The impact of such interactions between surface charge or organisms, surface and swab have not been assessed, and it has been suggested that other factors such as sampling pressure play an important role. As greater pressure is allowed with a less flexible swab shaft, selecting a swab with a less flexible shaft, such as wooden handled, may be a superior choice [263]. A looser swab bud has been proposed as a

reason for better sensitivity, as organisms could be more readily extracted from the swab tip into the collection medium [170]. More data is needed to assess how much this wrapping of the swab tip material may impact organism release, and if this has any significant impact on recovery.

Different surfaces also have different properties. One study found less transfer (to a meat exudate) of organisms from steel surfaces when compared to plastics. This reduction in transfer will result in poorer recoveries [316]. The attachment strength on plastic is greater than that from steel [316], leading to poorer recovery from plastic surfaces, which was reflected in the findings from this study.

The literature review of surface sampling devices (chapter 2) revealed the discrepancies and gaps in the literature. Table 2.4 highlights how much of the literature was missing, including many of the most important clinically significant pathogens. Due to the lack of data and different testing conditions, it was not possible to judge sampling devices equally and determine their efficacy under different conditions. It was clear that these devices needed to be tested individually, under a single, clear method, with different conditions. For this assessment, four pathogens that are recoverable from clinical surfaces and known to cause HCAI were selected for testing; *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis,* and *Klebsiella pneumoniae.* This provided two Gram-positive organisms and two Gram-negative organisms to form a representative comparison of potential surface organisms. These organisms were tested on ceramic, plastic, and stainless steel surfaces, of which make up the bulk of clinical surface types, as determined from a ward sampling experiment undertaken in chapter 6 [317].

3.2 RESEARCH AIMS

The aims of this chapter were to provide missing surface sampling data, to close some evidence gaps identified in chapter 2. This data would support surface sampling literature as a whole, as well as support surface sampling work undertaken in future chapters of this thesis. This was addressed by;

1. An in-house assessment of sampling devices found from the literature review; contact plates, petrifilms, dipslides, cotton and flocked swabs and sponges.
2. To assess these devices on a range of surfaces representative of the clinical environment (plastic, ceramic and steel) to identify how different sampling devices recover from different surface types.
3. To test four pathogens known to cause HCAI (*S. aureus, K. pneumoniae, E. faecalis* and *P. aeruginosa*) with all the sampling devices.
4. To assess and attempt to identify the impacts of surface type, pathogen and sampling device both individually and together, based on the results, to form conclusions on which sampling devices work best for different pathogens on different surfaces to help support decisions made by clinical staff trying to sample their environment.

3.3 METHODS

3.3.1 ORGANISM PREPARATION

Four different organisms were used within this study; *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (NCTC 12903)*, Enterococcus faecalis* (ATCC 29212)*, Klebsiella pneumoniae* (NCTC 13368). Organisms were grown from frozen stocks stored in beads at -70°C. A single bead was selected and aseptically transferred using a sterile plastic loop onto a tryptone soya agar (TSA) plate and grown at 37°C for 24-48 hours. A single isolated colony was selected and inoculated into 50ml tryptone soya broth (TSB) and grown at 37°C for 24 hours in an orbital shaker, at 150 rotations per minute. Following growth, cell numbers were determined using the Miles and Misra method [318].

3.3.2 SURFACE COUPON PREPARATION

Plastic and steel surfaces were provided and cut into coupons of 10x10cm^2 by the technical resources manager, CEGE, UCL. Ceramic bathroom tiles were sourced from B&Q and were provided at 10x10cm^2 from the manufacturer. To prepare the surfaces for inoculations, steel and ceramic surfaces were wiped down with 70% isopropyl alcohol to remove dust or debris. They were then wrapped in aluminium foil and autoclaved at 121°C for 30 minutes. Indicator tape was applied to the outside of the foil wrapping, and colour change was observed to ensure the 121°C was reached. Plastic surfaces were prepared by cleaning with 5% sodium hypochlorite, and the residue removed with 70% isopropyl alcohol.

3.3.3 SURFACE INOCULATION

Surfaces were allowed to fully dry before inoculation. Organisms were prepared as per the protocol defined in section 3.3.1. Each surface was divided into four sections, to produce four replicates. Each quarter section was inoculated with 100µl of bacteria in 5x20µl drops (cell concentrations 10^3/ml for direct methods to prevent overgrowth and 10^7/ml for indirect methods to account for processing losses). Surfaces were allowed to fully dry for 1.5 hours under laminar flow before sampling could commence.

3.3.4 SURFACE SAMPLING

3.3.4.1 DIRECT METHODS

Nutrient agar Petrifilms (3M, UK) were prepared by aseptically lifting the outer film and pipetting 1ml sterile deionised water into the center of the paper surface. The film was replaced and a plastic spreader was used to form a gel surface into a circle, producing the sampling surface. To allow this gel surface to solidify, the plates were refrigerated at 2-8°C for a minimum of 1 day before use, as per manufacturer instructions. Following refrigeration, the upper film was removed and the slightly adhesive lower sampling film was pressed onto the surface with firm pressure for 10 seconds. The upper film was replaced, and the sampling device incubated at 37°C for 48-72 hours. Tryptone-soya agar (TSA) contact plates (Thermoscientific, Basingstoke) and TSA dipslides (VWR, Leicestershire) did not need preparation. The lids were removed and the agar surface pressed onto surfaces for 10 seconds with firm pressure. The lids were replaced and incubated for 24-48 hours at 37°C. Colony forming units (CFU) were counted.

3.3.4.2 INDIRECT METHODS

Cotton swabs and flocked swabs (COPAN, USA) were briefly wetted with sterile water. Samples were taken from the surface by pressing firmly with the swab tip, rotating while sampling. Firm strokes were taken across the entire surface in horizontal, vertical and diagonal motions. Following sampling, the swab tip aseptically was transferred into a microcentrifuge tube containing 1ml sterile water, and vortexed for 1 minute to elute the sample from the swab tip into the diluent. 100µl of the diluent was plated onto TSA, spread with a sterile disposable plastic spreader, and incubated for 24-48 hours at 37°C, and CFU were counted.

Sponges (Hygiena, Watford) were supplied pre-wetted with buffered peptone water. They were aseptically removed from the packaging by only touching the handle of the sampling stick, and samples taken from the surfaces following the same protocol for cotton swab sampling. Following sampling, the plastic handle could be squeezed to aseptically release the sample collection sponge tip into the sterile collection bag. 50ml of phosphate buffered solution was added to the bag and agitated by hand for one minute to release the sample from the sponge into the diluent. 100µl of sample was pipetted and plated onto TSA, spread with a sterile spreader, and incubated for 24-48 hours at 37°C.

3.3.5 DATA ANALYSIS

Data were analysed using IBM SPSS statistics software version 26. Shapiro Wilk test determined normality. Data were transformed to proportional and analysed with general linear model with log regression. All surface samples were

completed with a minimum of 7 and maximum of 15 biological replicates, with the maximum replicates completed where possible.

3.3.6 ORGANISM RECOVERY CALCULATIONS

Recoveries were calculated as per methods used in similar studies [269, 297]

Sampling efficiency was calculated as per; SE = Ci/Ct x100

Recovery sensitivity was calculated as per; S = 100/ (A x SE)

In which;

SE = Sampling efficiency

S = Sensitivity

Ci= Concentration of organism recovered (CFU/cm^2)

Ct= Concentration of organism inoculated (CFU/cm^2)

A= Area tested in cm^2

Variance was calculated as: $$s^2 = \frac{\Sigma(x_i - \bar{x})^2}{n-1}$$

In which;

S^2 = Variance

x^- = mean CFU recovered

n = sample size

3.4 RESULTS

3.4.1 SURFACE SPECIFIC SAMPLING

When considering surfaces (N= 3) individually, for all organisms (N= 4) and all sampling devices (N=6), overall, ceramic surfaces (N=360 samples) were the easiest to recover the organisms from (23.84%), followed by metal (stainless steel) surfaces (N=335) (18.92%). Plastic surfaces (N=313 samples) proved a greater challenge for the sampling devices (14.80%). It was found that surfaces had a significant impact on recovery (P<.0001) though no statistical significance was found comparing plastic and steel (P=.699). Significance was found between ceramic and steel (P<.05)

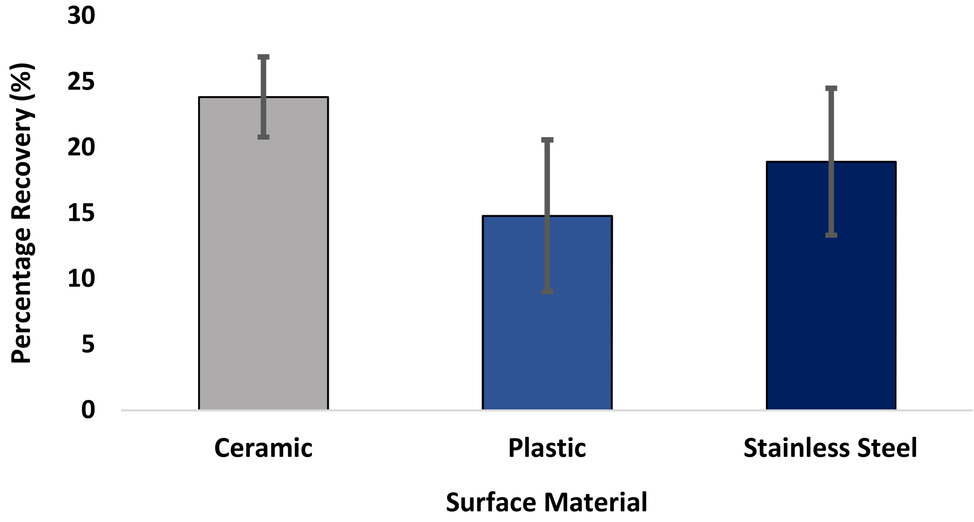


Figure 3.4 Average (mean) percentage recovery from ceramic, plastic and metal surfaces, for all organisms and surface sampling devices with error bars representing standard deviation.

3.4.2 PATHOGEN SPECIFIC SAMPLING

Target pathogen also had a role in recovery. Some pathogens were more readily recovered than others. The results found that, on average (mean), the Gram-positive organisms *S. aureus* and *E. faecalis* were more readily recovered (24.27% and 23.08% respectively) than the Gram-negative organisms *K. pneumoniae* and *P. aeruginosa* (20.01% and 11.57% respectively) though to no statistical significance (P=.718.).

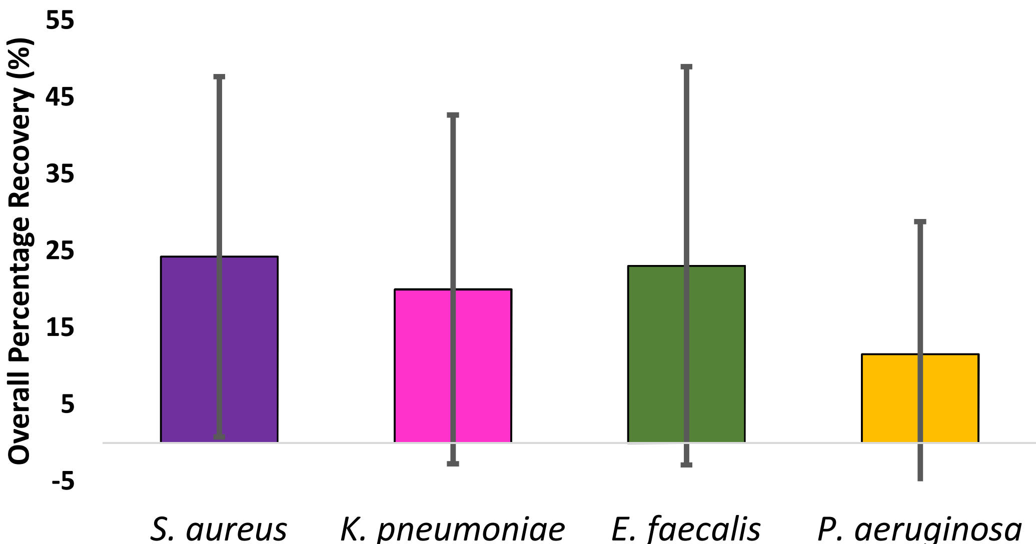


Figure 3.5 Average (mean) percentage recovery of four different pathogens with all surface sampling devices and all surfaces with error bars representing standard deviation.

3.4.3 INDIVIDUAL DEVICE SAMPLING

Overall, when assessing recoveries from all organisms (N=4) and all surfaces (N=3), dipslides and contact plates had the best recoveries (47.77% and 36.19%) respectively, and the difference between dipslides and contact plates was not statistically significant (P= .164). Petrifilms allowed, on average (mean), a 26% recovery. Sponges, flocked and cotton swabs performed poorly (0.23, 2.35, 2.55%). Between swabs, flocked and cotton swabs had no difference in performance (2.35-2.55%) P=.841. The difference between direct (contact plates, petrifilms, dipslides) and indirect methods (swabs and sponges) was significant P<.0001.

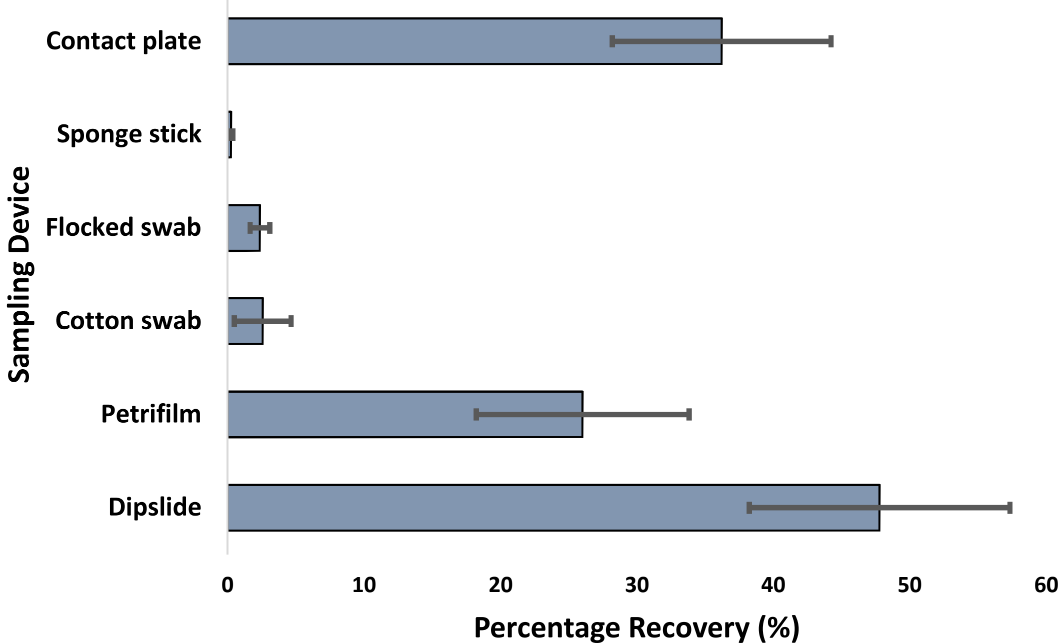


Figure 3.6 Average (mean) percentage recovery of all organisms from all surfaces recovered by individual sampling devices with error bars representing standard deviation.

3.4.4 INTERACTIONS BETWEEN SURFACE, PATHOGEN AND SAMPLING DEVICE

The results identified how sampling device, target organism and surface have an impact on recoveries. When sampling, these multiple factors work together to help or hinder the sampling process. When identifying the interactions between different pathogens and sampling devices, across all surfaces, it was found that different pathogens are more readily recovered using different sampling devices. For *K. pneumoniae*, contact plates (P= .385), dipslides (P= .243) and petrifilms (P= .002) were more effective, giving recoveries of 44.5, 50.5 and 26.2% respectively. For *P. aeruginosa*, dipslides (P= .011), petrifilms (P= .0001) and contact plates (P= .0001) were most effective, with recoveries of 30.9, 18.3 and 16% respectively. For *S. aureus*, dipslides (P= .946), contact plates (P= .379) and petrifilms (P= .005) gave the highest recoveries, at 50.5, 47.5 and 28.3% respectively. Finally, for *E. faecalis*, the most effective sampling devices were dipslides (P= .297), contact plates (P= .077) and petrifilms (P= .008), with recoveries of 59.2, 36.7 and 31.2% respectively.

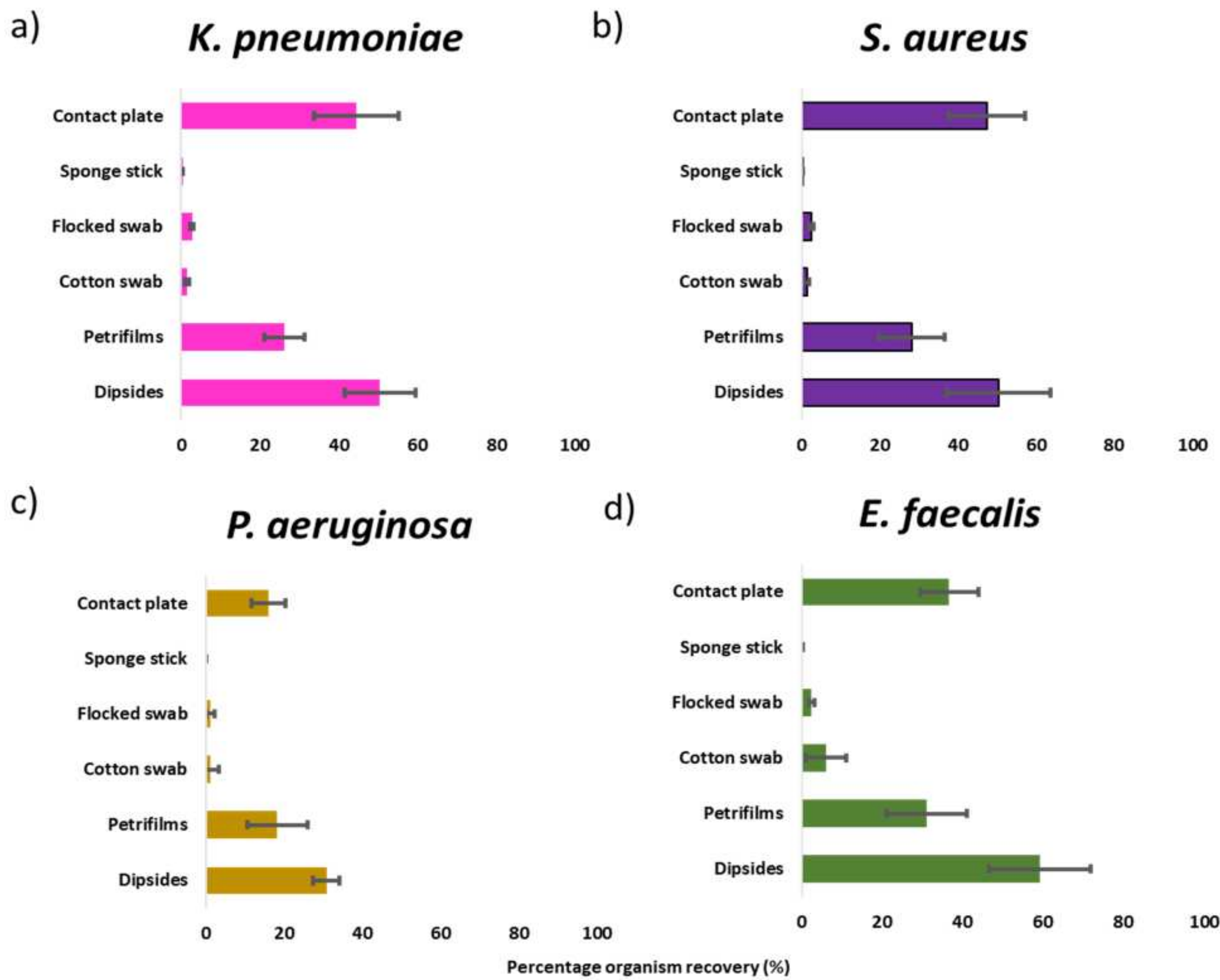


Figure 3.7 Average (mean) recovery of a) *K. pneumoniae* b) *S. aureus* c) *P. aeruginosa* d) *E. faecalis* using different sampling devices across all surface types with error bars representing standard deviation..

When considering the impact of recovering different pathogens from different surface types, it was found that that there was a difference in efficacy of recovery depending on the surface type and target organism. For ceramic surfaces, *E. faecalis* and *K. pneumoniae* (27.78 and 27.75%) were recovered most easily (P<.0001) compared to *P. aeruginosa* and *S. aureus* (21.42 and 18.42%). For steel surfaces, there were poorer recoveries overall, and here, Gram-positive organisms *E. faecalis* and *S. aureus* had the best recoveries (28.80 and 22.59%) compared to *K. pneumoniae* and *P. aeruginosa* (15.64 and 8.63%). For plastic surfaces, *S. aureus* and *K. pneumoniae* were more readily recovered (24.28 and 19.51%) and *E. faecalis* and *P. aeruginosa* had the poorest recoveries (11.49 and 3.92%). These results highlight the importance of surface material when selecting for a specific organism, and how recovery can be skewed due to the ability to recover some organisms more readily than others from different surface materials.

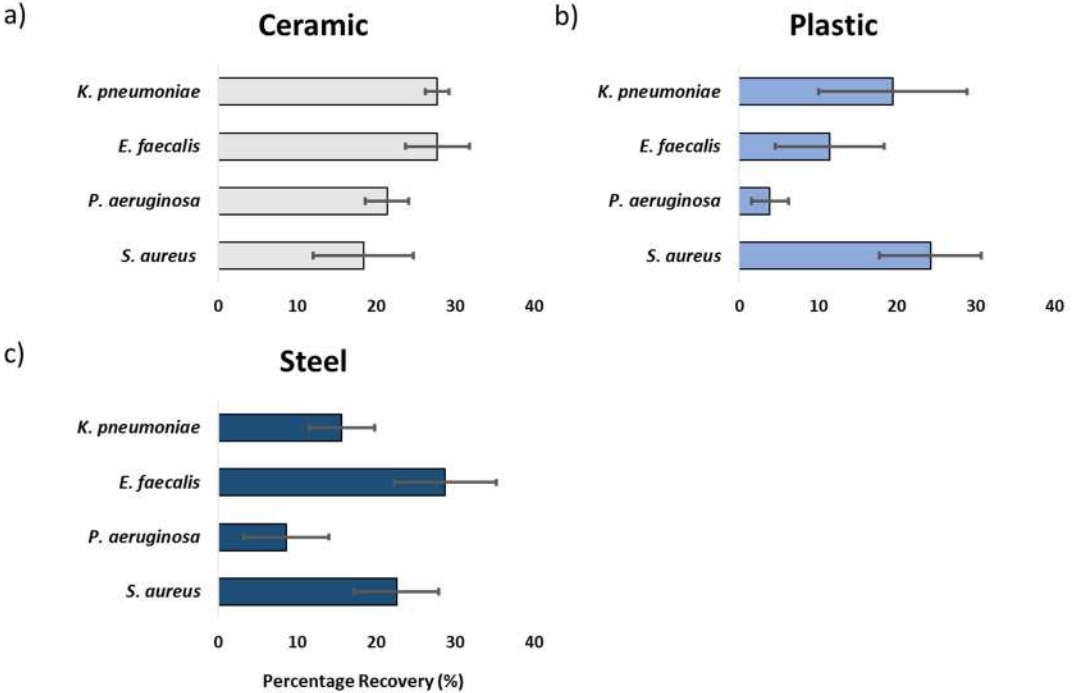


Figure 3.8 Average (mean) percentage recovery for all sampling devices from a) ceramic b) plastic and c) steel surfaces with different target organisms with error bars representing standard deviation.

Finally, when considering the interactions between sampling device and surface material, it was found sampling devices perform better on some surfaces than others. Dipslides performed best on ceramic surfaces (59.36%), compared with plastic (39.54%) and steel (43.79%). Similarly, for contact plates, in which 45.72% recovery was found on ceramic surfaces compared to 26.28% and 36.31% from plastic and steel respectively. These results were supported by the individual sampling devices and surface tests, in which different organisms had different recovery efficacy ($P < .0001$). Swabs and sponges still performed poorly overall.

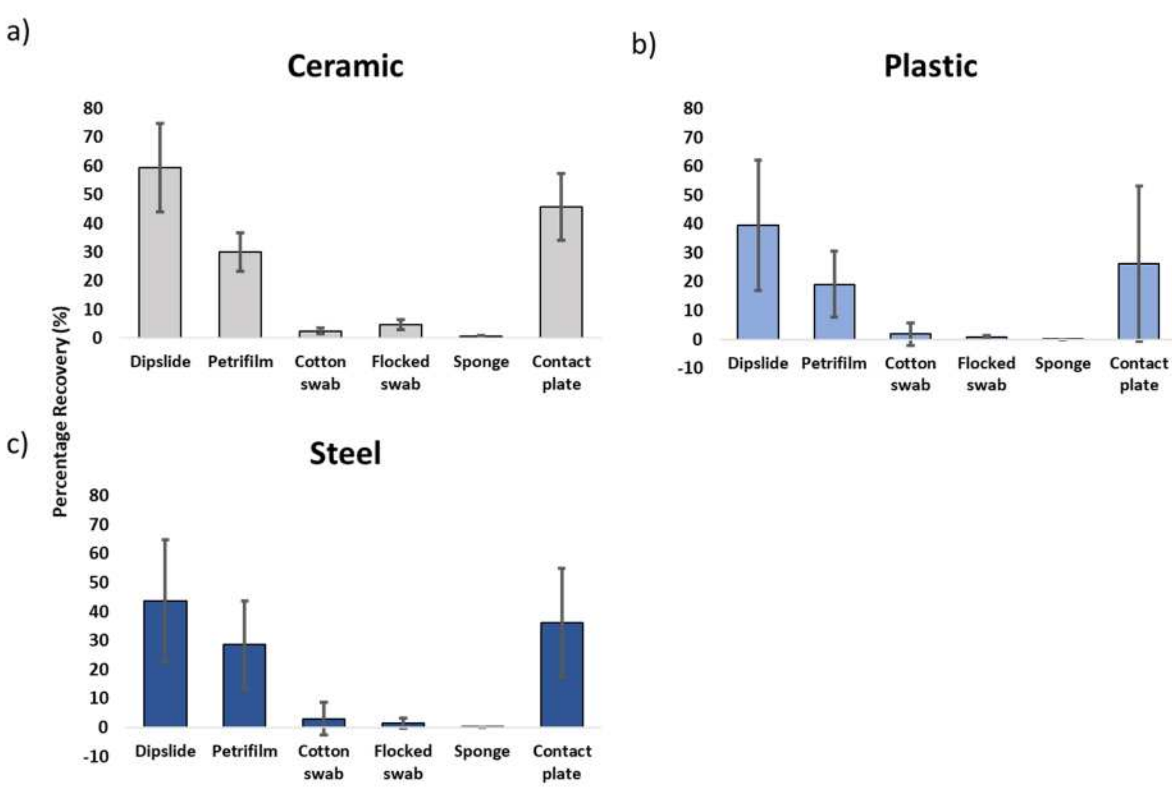


Figure 3.9 Average (mean) percentage recovery with different sampling devices from a) ceramic, b) plastic and c) steel surfaces with error bars representing standard deviation.

3.5 CHAPTER DISCUSSION

A large gap in the literature concerning surface sampling of clinical surfaces was identified. A wide range of sampling devices were tested, using varying methods and techniques, making a direct comparison between studies difficult. Often, the literature was contradictory and incomplete. Multiple gaps in the literature would make it difficult for a user to confidently assess different sampling devices, and to select a sampling device that would work well for their specific environment. A gap analysis in chapter 2, in regards to the most common clinical pathogens of concern, (*S. aureus, P. aeruginosa, K. pneumoniae, E. faecalis*) identified just how much data was missing. This study allowed a modest contribution to the literature.

3.5.1 THE ROLE OF SURFACE MATERIAL

The results identified how much of an impact surface materials can have on organism recovery. As the clinical environment is made of many different surface types and textures, these impacts are of concern when choosing a sampling device. Knowing how surfaces play a role in organism recovery is vital to make an informed choice when selecting sampling devices. The results identified that, overall, organisms were more readily recovered from ceramic surfaces, then metal, and proved most difficult to recover from plastic surfaces. Difficulty in recovering organisms from certain surfaces, such as plastics (figure 3.1) could be due to the ability of organisms to adhere and form biofilm on some surfaces more easily than others. Surface charge also plays a role in bacterial attraction, as negatively charged surfaces have been found to attract more bacteria than positively charged surfaces [298]. Therefore, surface materials play an important role in how well surface

sampling devices may perform, and surface material must be considered when making a selection between different surface sampling devices. For sampling devices with lower sampling efficiencies, such as swabs and sponges, the impact of such careful selection may be negligible, and potential improvement limited, and other factors may have a more weighted and significant impact, such as adsorption of cells [269] or the differences between wet and dry surfaces [268].

3.5.2 HOW DIFFERENT PATHOGENS ARE RECOVERED FROM SURFACES

It is important to consider that the clinical environment is inhabited by a multitude of both Gram-negative and Gram-positive organisms [99, 139, 219]. A study undertaken by Lemmen *et al.* (2004) found that multi-resistant Gram-positive organisms were recovered from ICU surfaces (26.4% positive samples) and general ward surfaces (23.6% positive samples) more frequently than multi-resistant Gram-negatives from ICU (8.1% positive samples) and general ward (2.6% positive samples) surfaces, likely due to Gram-positive organisms surviving better in air [99]. It is estimated that in the US, Gram-negative organisms are responsible for 30% of HCAI [319]. Both Gram-negative and Gram-positive organisms have varying abilities to attach and form biofilm on surfaces. Adhesion is aided by a number of components, such as an organisms use of flagella, chemotactic proteins, adhesins, liposaccharides and capsules [311]. Different bacteria may possess all or some of these components. While overall, ceramic surfaces were shown to give better recoveries, the results found that this varies depending on the target organism. For example, *S. aureus* had poorest recoveries on ceramic surfaces, and superior recoveries when compared with plastic ($P<.01$) and steel surfaces ($P=.094$). *E. faecalis* was recovered better from

ceramic and steel surfaces (P=.898), but was not recovered well from plastic surfaces in comparison (P<.001). To further investigate this trend, both size, shape and adhesion can be explored for these organisms

Table 3.1 Properties of organisms used for surface sampling efficacy testing.

	Size (µm)	Morphology	Adhesion	Stain	Reference
S. aureus	1	Cocci	'slime' production and ability to form biofilm	+ Gram Positive	[320, 321]
K. pneumoniae	1-2	Rod	Forms biofilm, has pili to aid adhesion	- Gram Negative	[322, 323]
E. faecalis	0.6-2	Diplococci	Can form biofilm	+ Gram Positive	[324, 325]
P. aeruginosa	1.5-3	Rod	Forms biofilm, has pili, strong colonising capacity	- Gram Negative	[316, 326, 327]

Table 3.1 above shows how each organism varies in size, shape, and the mechanisms they have at their disposal for surface attachment. These factors will play a role in how easily they can attach to fomites, how they survive in the environment, and how they can be recovered from different sampling devices. Some cells can adhere faster to surfaces; the attachment of *P. aeruginosa* to stainless steel

showed that cells in early or late log phase adhered in twice the numbers as cells in stationary phase [309]. In the context of hospital surfaces, organisms are not likely to be actively dividing as the surface environment (unless soiled, or organic matter is present) do not provide a nutrient rich environment. However, it has been suggested that low-nutrient environments give greater adherence to surfaces as the stress allows more expression of cell surface characteristics in relation to finding nutrients, which allow an organism to become more closely associated with a surface while trying to find nutrients, though these results were suggested against tests with marine *Pseudomonas* spp. And *Vibrio* spp. Therefore implications against clinically significant pathogens may be limited [309, 328]. Pili and flagella were found to have the greatest impact on retrieval from surfaces with varied topography [311], highlighting how both surface factors and bacterial properties can interact and have an impact on recovery. When assessing both surface type, recovery, and surface device efficacy, the following conclusions can be made in table 3.2 below on how easily different pathogens can be recovered from different surface types, tested under a single environmental condition. As demonstrated by the review of surface sampling devices (Chapter 2) there is a range of conditions that can impact recovery using a sampling device (surface material, porosity, topography wet or dry surface, level of bioburden on a surface, cells being adsorbed, stressed or damaged, soiling presence, use of wetting agent and type of agent used and transport medium used and storage conditions). Optimisation of sampling technique, use of different sampling device or testing under different environmental conditions may yield different results, and surface sampling devices such as swabs, with known poor

sampling efficiency are not likely to show significance across different surfaces or organisms as efficiency is limited.

Table 3.2 Ease of recovering different organisms from different surface materials as suggested by the results.

	Steel	Ceramic	Plastic
S. aureus	√!	√!	√
K. pneumoniae	X	√	√!
E. faecalis	√	√	X
P. aeruginosa	X	√!	X

Key	
√	good recovery from surface
√!	Moderate recovery from surface
X	Poor recovery from surface

Regardless of target surface or sampling device used, some organisms are more easily recovered than others. It has been shown that different strains of the same organisms have differences in recovery [272, 283], which means, even with careful sampling device selection, there will still be a natural variation in how easily some organisms are recovered. This is important to consider when sampling environments to build up a picture of the microbiome. Easy retrieval of certain species may skew the results when trying to determine the levels of different organisms inhabiting the surface environment. Some organisms are notoriously difficult to recover, either due to the difficulty replicating their growth conditions or general low numbers present on surfaces, while others are viable but non-culturable [107, 108]. As such, environmental monitoring can only provide insight into a select few organisms. As discussed in Chapter 1, there are both traditional microbiological

analysis methods as well as molecular analysis. From the sampling devices tested, only swab sampling devices are suitable to undergo both traditional and molecular testing. Molecular methods do not require organisms to be in a culturable state, and can therefore be more sensitive. Furthermore, molecular analysis can be an indispensable tool when tracking pathogen spread and acquisition between patients and the environment, as well as locating the source of the outbreak.

To put these findings into practical context, it is important to consider how all these factors will play a role in the real clinical surface environment. The surfaces chosen for this research were new and undamaged. Surfaces in the clinical environment could be decades old, and will have associated wear and tear from daily use, leading to additional microscopic damage, allowing more surface area for organisms to enter and hinder recovery with sampling devices. Inappropriate use of cleaning equipment could also contribute to surface wear and damage. Furthermore, clinical surfaces will be made of a variety of materials. An effective routine sampling of a single room will consist of several material types, therefore choice of sampling device must be considered carefully. Another important factor is the environmental stressors the organisms will face. Harsh environments can cause organisms to express different genes to aid survival and allow strong surface attachment and biofilm formation. Strains that have survived in the clinical environment over time will undoubtedly be recovered differently from lab-based studies, in which freshly grown pathogens have been inoculated onto new, single surfaces. In clinical environments, biofilms are formed of many different species that can both help or hinder each other [329]. The interactions between several species on a surface can

have an effect on survival. These interactions could also play a role in adhesion abilities, and cause a change in how well certain species are recovered from different surfaces.

3.5.3 SURFACE SAMPLING WITH DIFFERENT DEVICES

Surface type and target organism aside, it has been shown that different surface sampling devices will always perform differently (figure 3.5). When assessing all surface types and target organisms, contact plates and dipslides performed well, with recoveries of 47.77% and 36.19% respectively. Petrifilms had a 26% recovery, and sponges and swabs performed poorly, not exceeding 2.55% recoveries. Some devices, for the pathogens considered within this study, were found to consistently perform poorly, such as flocked swabs, cotton swabs and sponges. However, this was on a select few pathogens. Sponges have been found to be very successful for recovering *C. difficile* from surfaces [277], potentially due to the spore shape and the sponge texture allowing easy recovery. As previously discussed in chapter 1, surface sampling devices perform differently under different surface conditions, such as contact plates performing poorly on wet surfaces, or surfaces with a high bioburden (chapter 1). Direct contact methods had lower variability between samples. Average variance for contact plates (s^2 =134.16), dipslides (s^2 12.52) and petrifilms (s^2 10.78) compared with indirect methods, sponges (78.80) cotton swabs (303.80) and flocked swabs (192.20). This is unsurprising between swab and non-swab methods, as swabs are difficult to standardised as a large portion of sampling relies heavily on the individual technique used, pressure, and number of strokes. While pressure can be variable with agar-based direct samplers, like contact plates, these are easier to

standardise. Between the direct samplers, dipslides had lower variance compared with contact plates, suggesting the design or use of dipslides is a more reproducible technique, or that the manufacturer of the sampling device is more uniform. Compared with the swab and sponge samples, sponges had lower variance than the two swabs. This may be due to the size of the sponge tip and handle, which are significantly larger than the cotton or flocked swabs, therefore it is easier to see if the sampling technique is consistent between samples. Between the swabs themselves, flocked swabs had lower variability compared with cotton. This has been explained by the material difference and interaction with the sample, as cotton fibers have many wrinkles and folds which increase surface area of absorption which will allow greater pickup of sample, but the attraction of water molecules to the cellulose rings in the cotton could lead to entrapment of bacterial lamellae and will become trapped in the swab bud. In comparison, the flocked swab coating will prevent the bacterial suspension from becoming entrapped in the matrix of the bud, allowing more successful vortexing and release of the sample [263]. Dolan *et al.* noted during testing different swab types, there was a difference in the bud tips, and the neutralising buffer swabs were wrapped more loosely around the handle compared with cotton swabs. This means the neutralising swabs may have had easier release of organisms from the bud tip into the enrichment media, giving the suggestion of higher sampling sensitivity, and that this may be a factor allowing better sensitivity (2.6×10^{1}) compared with the other swabs tested on the bench surface eSwab (6.1×10^{-1}) macrofoam swabs (3.9×10^{-1}) saline swabs (2.8×10^{2}).

With these factors in mind, and consideration of the interactions between target organism and surface type having an impact on recovery, the ability for a sampling device to recover an organism goes far beyond that replicated in a laboratory environment. The results highlight that there is no single sampling device that will work for all environments, and all factors, including the efficacy of the sampling device itself, must be considered during selection.

3.6 **CONCLUSIONS**

It has been shown that there are many factors that can either help or hinder the recovery of different organisms from different surfaces. Some surfaces allow easy recovery of pathogens, whereas other surfaces prove more difficult. Many factors are at play, such as the surface texture, surface charge, and any potential microscopic damage to the surface. Furthermore, the organism type also plays a role in recovery, and some organisms are more readily recovered. Size, shape, adhesion ability and how quickly a species can form biofilm could all play a role in how well it can be recovered from a surface. However, perhaps the most important component of surface sampling is ensuring the choice of surface sampling device is appropriate to the situation. Chapter 2 revealed the gaps in literature in relation to surface sampling device testing. This chapter sought to build on the evidence and add to the literature assessing clinically significant surfaces (metal, ceramic, plastic) against clinically significant pathogens (*S. aureus*, *K. pneumoniae*, *E. faecalis*, *P. aeruginosa*) and highlight the need for more testing of surface sampling devices, including those not affiliated with the clinical environment and testing for clinical pathogens, such as petrifilms and dipslides,

Different sampling devices have varying limits of detection. Sponges and swabs consistently performed poorly, whereas dipslides and contact plates were shown to be the most effective. It is critical to ensure all these factors are considered when selecting a device for surface sampling, and careful consideration of target organism and the type of surfaces to be sampled must be considered. While all these factors have been shown to play a role in pathogen recovery, it is vital to consider how

different real clinical environments are. The results from this study give important information and guidance for application in the clinical surface environment. However, it is important to consider that lab-based studies cannot account for the different organic factors that are at play in clinical environments, and often result in a ‘best case’ scenario result, as is common in lab-based research.

Now the options for surface sampling have been identified, while there are still gaps in the literature, these sampling devices can be used across different settings to assess how well cleaning has been undertaken, or to determine patient risk. Surface sampling allows a quantitative or semi-quantitative assessment of how well cleaning has been undertaken. Chapter 4 will explore cleaning.

Chapter 4 CLEANING – A REVIEW OF THE LITERATURE

4.1 INTRODUCTION

Cleaning is the physical removal of dirt, debris, general soiling and infectious materials, and is defined by the CDC as 'the removal of foreign material (e.g. soil, and organic material) from objects and is normally accomplished using water with detergents or enzymatic products' [330]. Removal of any visible soiling is the first step for cleaning, to physically remove and break down organic matter, which can be followed by a further step with a disinfectant if required. By cleaning hospital surfaces, the risk of the transmission of infectious organisms is reduced. The nature of the clinical environment and presence of infected and colonised patients means the surface environment can easily harbour these pathogens [196]. Effective cleaning can ensure the surface environment is safe for patients, and reduces this risk [331].

Despite the importance of cleaning, there can be confusion as to the type of cleaning undertaken and the definition of the word clean. The term cleaning refers to the initial step all cleaning efforts, regardless of level, must follow, which is the physical removal of any physical dirt, dust or soiling, as disinfection cannot occur through soiling. Decontamination is the removal of soil or pathogenic organisms in order to make an object safe for handling, prior to further processing or discard, such as for the reprocessing of medical devices [332]. Disinfection and sterilisation are not synonymous and represent two very different cleaning procedures, depending on the criticality and purpose of a surface. Disinfection reduces the amount of organisms from an object or surface, usually measured as a 5-6 log_{10} reduction as tested under

controlled conditions [330]. Surface cleaning of hospital environments is a complex undertaking, as there can be multiple confounding factors that may make cleaning agents less effective, such as the presence of soil on a surface which would inactivate a QUAT-based cleaning agent, as they are readily deactivated in the presence of soil, whereas other methods are more suitable for the use in presence of soil, such as peracetic acid. Peters *et al.* 2018 detail these conditions using the acronym W.A.S.T.E; workforce (individuals responsible for the cleaning activity), area (environment that is to be cleaned, including surface type, if the surface is intact and the level of cleaning required), substance (the chemical or product that is to be used for cleaning, if this is a disinfectant or detergent), technique (the method used for cleaning), equipment (the equipment used for cleaning be this manual with a microfiber cloth or touch-free like hydrogen peroxide vapour systems) [333].

All these variable conditions are difficult to replicate in efficacy testing, therefore the required 5-6$\log_{10}$ reduction may not be achievable in real-life settings. This is not always achieved when tested under conditions more closely related to those that may be present within the clinical environment. Limitations may be from user error, such as incorrect dilution of cleaning agents or using contaminated cleaning agents which can lead to microbial seeding of surfaces [4] or limitations of the cleaning agent itself. In relation to surfaces, surface material plays a role in the efficacy of the cleaning agent. Scratched surfaces (polyethylene, polypropylene, glass, and stainless steel) had reduced log reductions of *S. aureus* in comparison to non-scratched counterparts when cleaning with sodium hypochlorite [334]. The degree of scratching on individual surfaces is difficult to replicate in laboratory

testing conditions. Hospital environments vary, and surfaces differ in age across different settings. This thesis tested at GOSH, which is a historical institution and was founded in 1852. The hospital is formed of older, historical buildings and newer, up to date wards. Older surfaces are more likely to have significant scratching and degradation. In addition, hospital in developing countries may not have the resourcing for replacing worn out or old surfaces. Therefore, it can be difficult to predict how a cleaning agent will perform in clinical settings, therefore careful selection is key depending on the visible factors that can be assessed prior to undertaking cleaning (W.A.S.T.E).

Sterilisation is the total kill of all organisms, including spores. Sterilisation is often undertaken using heat or steam, by autoclave or other similar devices, and is effective for medical devices and other critical heat-proof instruments, in which all organisms are destroyed. Disinfection is the cleaning of surfaces and devices, using high-level disinfectants or lower level disinfectants, depending on the level of clean required (intensive and frequent cleaning with high-level disinfectants for high risk areas, or less frequent, though still regular, cleaning and spot cleaning as required with lower-level disinfectants or detergents) [192]. Intermediary (medium-level) cleaning can be undertaken with less concentrated cleaning agents. Low-level cleaning would be undertaken within the general hospital and communal areas, whereas high-level cleaning would be used for terminal room cleaning, and for cleaning during an outbreak, or a room exposed to an infected or colonised patient.

An antimicrobial is defined as an agent that kills or stops the growth of microorganisms [335]. Some antimicrobial cleaning agents are targeted towards

bacteria, such as bacteriocides, and others target fungal organisms, such as fungicides. Different cleaning agents work in different ways; some kill bacteria (bactericidal) while others do not kill bacteria, but instead prevent their reproduction (bacteriostatic). Virucidal agents will destroy viruses. For a higher level clean, a cleaning agent must be selected that can kill spores, called a sporicidal agent. Some cleaning agents, such as no-touch automated room disinfection systems like hydrogen peroxide 'robots', are to be used as an adjunct to cleaning only. The process of understanding all these terms and where they should be applied and their implications for clinical environments can be difficult, and is only worsened by the lack of available guidance and standards for clinical surface cleaning. Cleaning procedure, training, re-training, competency, choice of cleaning agent and frequency of cleaning vary in different healthcare settings and protocols are set by each individual setting. Cleaning training packages and local procedures are often not based on evidence, [208, 336] and there is a distinct lack of robust and complete guidelines for cleaning [200, 336].

Failures in cleaning compliance have been documented well in the literature [159, 197, 198], and poor cleaning is known to increase the risk of HCAI. Yet assessing cleaning is difficult, and currently there are no evidence-based methods to assess how well an individual cleaner has cleaned [212]. Currently, the available options for assessing surface cleanliness is by using the visibly clean audit, which is mandated for all UK NHS trusts [337], ATP testing, UV marking of surfaces to visibly locate missed surfaces, or microbiological sampling. Microbiological sampling is the superior method, though, again, there is a lack of evidence-based standards for assessing

clinical surfaces, and what would define them as ‘clean’ as visibly (aesthetically) clean and microbiologically clean (no pathogenic organisms) are different criteria, representing a different risk to a patient [139, 171].

It is clear cleaning is important to reducing the burden of HCAI, yet there are so many options. Selecting a cleaning agent and using the correct technique for different surfaces can be confusing.

4.2 RESEARCH AIMS

The aim of this research was to review the literature surrounding surface cleaning, and to compile the available information on different cleaning methods that can be used for clinical surfaces, and their potential applications. The review would allow a comprehensive analysis of possible cleaning options over a wide and varied amount of literature, to compile and present in a legible, accessible and useful format for healthcare professionals.

The following factors were considered;

1. What are the options for clinical surface cleaning?
2. What recommendations can be made for low (general), intermediary (medium) and high level cleaning?

4.3 METHODS

A systematic review was undertaken following a modified version of the PRISMA guidelines [214]. Study eligibility was determined using the EPIC guidelines [338], in which studies must rank either 1++, 1+, 2++ or 2+ to be accepted within this review.

The following online repositories were searched; ScienceDirect, Web of Science and Medline (PubMed). The keywords included; hydrogen peroxide vapour AND efficacy AND surface; sodium hypochlorite AND efficacy AND surface; peracetic acid AND efficacy AND surface; quaternary ammonium compound OR QUAT AND efficacy AND surface; microfiber cloth OR microfiber cloth AND efficacy AND surface; detergent AND efficacy AND surface; disinfectant AND efficacy AND surface; UV OR UV-C AND efficacy AND surface; sodium dichloroisocyanurate OR NaDCC AND efficacy AND surface; chlorine dioxide AND efficacy AND surface; wipes AND efficacy AND surface; chlorine AND efficacy AND surface; mops AND efficacy AND surface; ozone AND efficacy AND surface. Reference lists of included literature were searched in order to ensure full coverage. Studies were excluded based on the criteria outlined in table 4.1 below

Table 4.1 Inclusion and exclusion criteria for study review.

Inclusion	Exclusion
English language	Non-English
Results focusing on surface cleaning in clinical environments and in vitro	Results from efficacy testing with organisms in suspension
All surfaces, both clinical and non clinical and from other industries	Organic surfaces such as fruit and vegetables, and dental surfaces
All organisms of human significance, or appropriate surrogates	Animal pathogens
Evidence based papers with results	Opinion papers with no results

All organisms of human significance or their related surrogate organisms and non-organic surface types, both clinical and non-clinical, were included. Literature assessing organisms only in suspension were excluded. Literature assessing both surface and organisms in suspension were included, but only surface results were considered within this review.

4.4 RESULTS

Eligible literature was searched and assessed by title, abstract, then full-text content and adherence to the EPIC criteria shown in figure 4.1. N= 18,741 studies were retrieved from the keywords search. Following removal of duplicates and exclusion at title-level, N= 1,057 studies were reviewed at abstract level. N= 666 full-text articles were reviewed, and N= 426 were excluded using the inclusion and exclusion criteria as outlined in table 4.1. A total N= 240 studies were included within this review.

Only 1.2% of studies from the initial keywords search fulfilled the criteria as set out within this review. N= 168 studies were excluded as they assessed only organisms in suspension and not organisms present on surfaces.

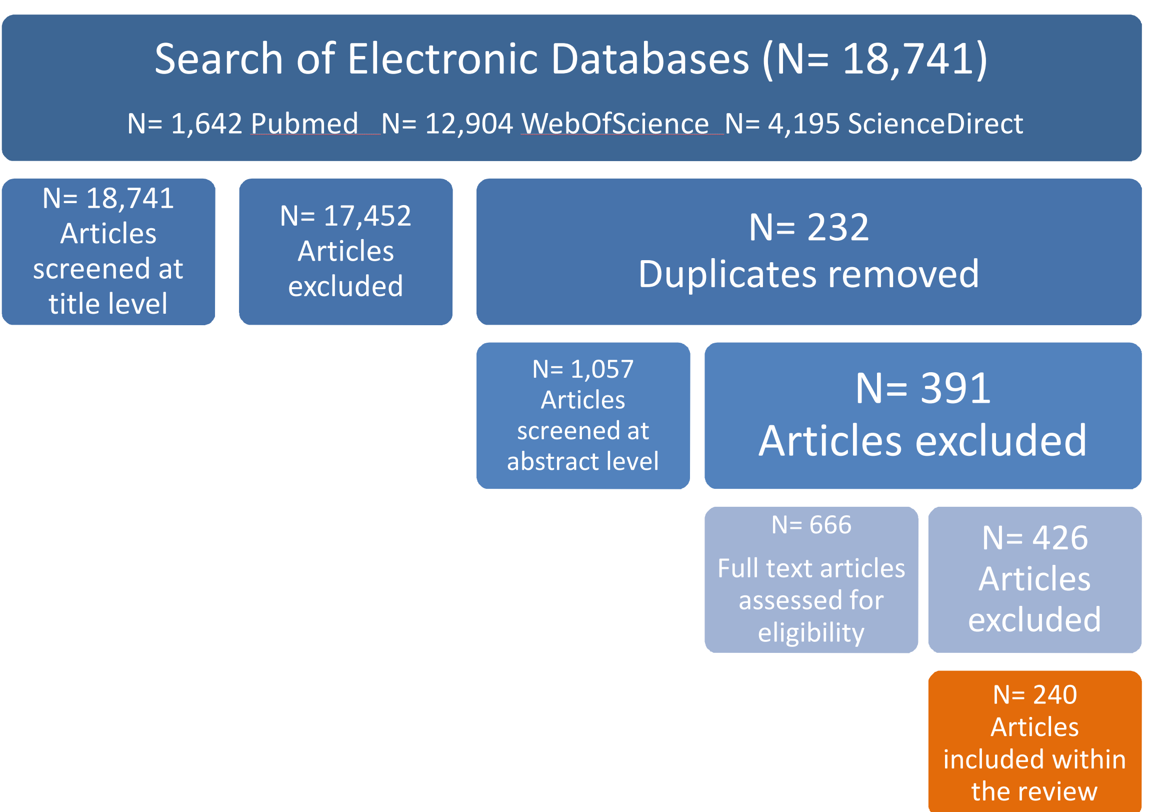


Figure 4.1 Diagram of the study review and selection process

Figure 4.2 details the studies excluded from the full-text review, and the reasons for exclusion. N=240 studies fulfilled the inclusion criteria and were included within the review. Within the studies selected for the review, N=34 were assessing cleaning within the hospital environment, N=197 were lab-based studies, N=6 were assessing both environments, and N= 3 were in other settings. A total of 12 groups of cleaning agents were identified; alcohols N=31, aldehydes N=18, oxidising agents N=36, acids and bases N=45, phenolics N=12, quaternary ammonium compounds (QUATS) N=63, chlorines N=82, iodine N=14, terpenes N=4, UV N=17, wipes, cloths and mops N=42, other and novel N= 29. A full breakdown of the components within each category is available within Appendix.

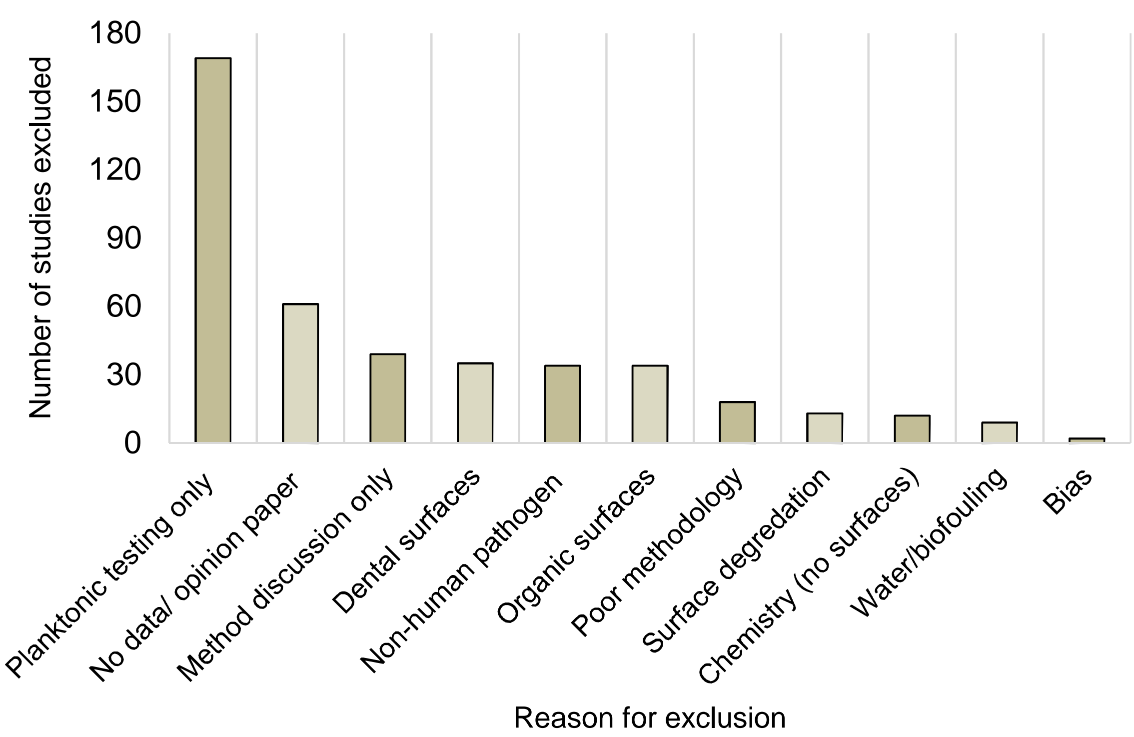


Figure 4.2 Number of studies excluded within the full-text assessment and the conditions of exclusion

4.5 RECOMMENDATIONS FROM THE REVIEW

The literature review revealed many options for surface cleaning, with varying efficacy, cost, and ease of use. Several cleaning agents, while effective, were classified as not appropriate for routine cleaning, due to the production of fumes, difficulty to use, or the staining or degradation of certain surface materials. Examples of such include cleaning agents that create fumes, like >400ppm chlorine, or cleaning agents that are highly irritating and toxic which include aldehydes. Peracetic acid and sodium hydroxide are not compatible with metal surfaces and are corrosive (see table 4.2-4.3). For high level cleaning, an agent must be able to destroy all organisms, except for high concentrations of spores, though the level of spores classified as high concentration is not defined [192]. The review identified the following suitable agents for high, moderate and low level cleaning:

Table 4.2 Cleaning Agents suitable for high level cleaning

Name	Mode of action	Advantages	Limitations	Potential of resistance?	Suitable for	Studies reviewing
Hydrogen peroxide >7%	Bactericidal. Produces free radicals that can attack membrane lipids, DNA, and other essential cell components	Powerful, no odour	Incompatibilities with some surface materials. Corrosive to rubber, brass, stainless steel and other metals	no	Some surface materials	N= 25
Hydrogen peroxide vapour (>300ppm)	Bactericidal. Produces free radicals that can attack membrane lipids, DNA, and other cell components	No residues, no disposal of chemicals required, no-touch system requiring no operator	Adjunct only – must be used following traditional surface cleaning	no	Entire room disinfection	
Chlorines (>400ppm)	Bactericidal. Denaturing proteins and enzyme-structure destruction	Lower concentrations are sporicidal, cheap, no known resistance mechanisms, a >4 log reduction demonstrated even for prions	PPE must be worn, odour, irritant to skin and eyes (at 400ppm)	no	All surfaces	N= 82
Peracetic acid (>500ppm)	Bactericidal. Denatures proteins, disrupts the cell wall permeability	By-products are environmentally friendly, sporicidal, works in presence of soiling, works well against spores	Incompatibilities with some surface materials	Yes, evidence of acid-resistant mechanisms	Surfaces except metal	N= 45 (all acids)

For medium or intermediate level cleaning, cleaning agents must demonstrate the ability to kill bacteria, mycobacteria, most viruses and fungi, though not spores [192].

Table 4.3 Cleaning Agents suitable for intermediary-level cleaning

Name	**Mode of action**	**Advantages**	**Limitations**	**Potential of resistance?**	**Suitable for**	**Studies reviewing**
Chlorine-based agents (<400ppm)	Bactericidal. Denaturing proteins and enzyme-structure destruction	Lower concentration solutions produce less odour, no known resistance mechanisms	Lower concentrations not sporicidal	no	General surface cleaning	N= 82
Alcohols (>70%)	Bactericidal. Denatures proteins	Readily available in wipes or sprays, cheap, fast drying	Drying to skin, can degrade some surfaces, fast drying which can make adherence to contact times difficult	Yes, evidence of gene mutation in alcohol-tolerant strains	General surface cleaning of non-rubber surfaces	N= 31
QUATS (>400ppm)	Bactericidal. Inactivates enzymes, denatures proteins, disrupts cell membrane	Some limited efficacy against viruses, cheap	Readily inactivated in the presence of soiling, not effective against spores, general cleaning must occur first	Yes, evidence of upregulation of efflux pumps	General cleaning	N= 63

Low level cleaning can destroy bacteria in a vegetative state, some fungi and viruses, but not mycobacteria or spores [192].

Table 4.4 Cleaning agents suitable for low level and general cleaning

Name	**Mode of action**	**Advantages**	**Limitations**	**Potential of resistance?**	**Suitable for**	**Studies reviewing**
QUATS (200-400ppm)	Bactericidal. Inactivates enzymes, denatures proteins, disrupts cell membrane	Some limited efficacy against viruses, cheap	Readily inactivated in the presence of soiling, not effective against spores, general cleaning must occur first	Yes, evidence of upregulation of efflux pumps	General cleaning	N= 63
Detergents	Can be bacteriostatic. Physical removal, breakdown of biofilm.	Environmentally friendly, no risk of antimicrobial resistance, cheap and readily available, remove soiling and organic matter	Physical removal of organisms only – no active ingredient to kill pathogens.	No, as no kill action exerted	General surface cleaning prior to disinfection. General cleaning of 'low risk' surfaces, such as communal areas.	N= 42

When using a cleaning agent, various application methods are possible with cloths, mops or wipes, all of which are made of different materials and suitable for different types of cleaning.

Table 4.5 Cleaning agent application methods

Name	Mode of action	Advantages	Limitations	Potential of resistance?	Suitable for	Studies reviewing
Cloths	Physical	Microfiber weave helps trap and retain organisms, cheap and disposable.	Transfer to other surfaces, no antimicrobial action without cleaning agent, traditional 3-folded method of cleaning proven to contaminate subsequent surfaces. Non-microfiber cloths have poor retention of organisms.	n/a	General cleaning	N= 19
Mops (for floor cleaning)	Physical	Can apply cleaning agent easily to large surfaces, can be microfiber, cheap, contaminated mop heads easy to replace.	Use across too large a surface can transfer contamination.	n/a	General cleaning	N= 5
Wipes	Physical (varies by specific active agent)	Fast and easy to use. Readily available, user friendly with no preparation required. Active agent pre-impregnated.	Can become contaminated and transfer pathogens, can dry out.	Variable to active ingredient	All smaller surfaces	N= 18

4.6 DISCUSSION

Cleaning is a critical component of IPC, to keep surfaces free of pathogens that could lead to acquisition of HCAI. Effective cleaning allows the clinical environment to remain safe, and reduce the risk of HCAI. Despite the importance of cleaning, cleaning protocols vary widely between different trusts. The review identified 240 studies assessing the efficacy of cleaning agents for surface cleaning, encompassing 12 groups of cleaning agents. However, more data is needed to support decisions faced by healthcare professionals when selecting cleaning agents, as the clinical environment is a variable and complex space, and cleaning agents must be tested rigorously under various conditions, such as on different surface materials, to provide the evidence for producing true evidence-based cleaning protocols. The variation in controlled studies, which has been highlighted in the literature, shows how efficacy must be demonstrated in field conditions starting with, for example, priority pathogens under a single method [339]. Using synergistic lab-based studies followed by in-hospital use and efficacy testing on clinical surfaces could start providing insight into how laboratory based efficacy translates under different testing conditions, and how this then relates again to in-house use in the hospital environment, in relation to presence-absence of clinically significant pathogens, general cleaning efficacy (TVC or other methods) and most importantly, how this correlates with HCAI incidence.

For cleaning clinical surfaces, surfaces are divided into categories of risk. The Spaulding classification has long been used as the gold-standard guideline for categorising risk, conceptualised in 1957, ranking surfaces as critical, semi-critical or

non-critical [88]. Hospital surfaces, as they do not come into contact with broken skin, are classified as low risk, and non-critical. This classification suggests intermediate (medium) or low-level cleaning processes are to be used here [88]. The national specification for cleanliness in the NHS follow a similar breakdown of surfaces divided into categories by risk. The national specifications for cleanliness in the NHS categorise areas in the following format:

High Risk

Cleaning standards must be consistently high, achieved by intensive and frequent cleaning. Areas in which invasive procedures are performed are included here.

Medium Risk

Cleaning standards must be good, to achieve both hygienic and aesthetic results, though not the same intensity and frequency as high risk areas. Regular cleaning supplemented with spot cleaning will maintain this standard.

Low Risk

The risk to patient safety in these areas is classified as low, therefore cleaning these areas is deemed aesthetic. Cleaning should be regular, though less frequent than medium risk areas, with minor spot cleaning when required.

Figure 4.3 Classification of high, medium and low risk areas for surface cleaning as per the national specifications for cleanliness in the NHS (NHS 2007).

Even the choice of cleaning agent can be a difficult and confusing choice for healthcare professionals. The choice between disinfectants, detergents, or the use of both is heavily debated, as both have their own inherent advantages and limitations. Detergents do not have the risk of encouraging resistance, as they have no kill action against organisms unlike disinfectants [340], are more environmentally friendly, often safer for the user, and are easy to dispose of. However, they act with physical removal alone, and can become easily contaminated if not used or stored correctly [3].

Detergents are an extremely important part of cleaning. They can be broken down into the following categories; anionic detergents, cationic detergents and non-ionic detergents [341]. Anionic detergents, such as sodium cholate or sodium deoxychloate-based detergents, are synthetic detergents that have their cleaning activity regulated by the anionic portion of the molecules. They can contain the following active ingredients; sodium alkyl sulfates, sodium lauryl sulfate and sodium alkyl benzene sulphonate [342]. When added to water, anionic detergents ionize and have a negative charge [342]. They are very effective at cleaning in the presence of soiling [341]. Cationic detergents can contain the following ingredients; chlorides, bromides, acetates. They are most commonly used as fabric softeners or in the cleaning of textiles, and are more expensive, and are not used for clinical surface cleaning. Non-ionic detergents can form hydrogen bonds with water, and have a neutral group in their molecules. They are monoesters of polyhydric alcohols or polyethers, derived from ethylene oxide [341]. They are effective at stabilising emulsions [341]. Non-ionic detergents do not carry a charge in the head of the

surfactant molecule and have lower water solubility compared with ionic detergents. Some environmental surface cleaners can be a blend of different types of detergent, to increase the efficacy and gain the advantageous properties across both, such as combination nonionic-anionic blend like the HC90 neutral detergent which has been used in hospital cleaning [343].

Detergents do not kill organisms, but break down organic matter and dirt on surfaces and remove organisms with a physical action applied by a cloth or mop. This can then be followed by a disinfectant if required. Detergents do this by their formulation, made up of a surfactant or a combination of multiple surfactants. Surfactants, or surface active agents, are molecules that bond together to form bubbles. The hydrophobic end of the molecules are attracted to the soiling, binding the soiling (figure 4.4A). The other hydrophilic ends of the molecules are then attracted to water (figure 4.4B), and are then removed with the damp cloth or mop [344].

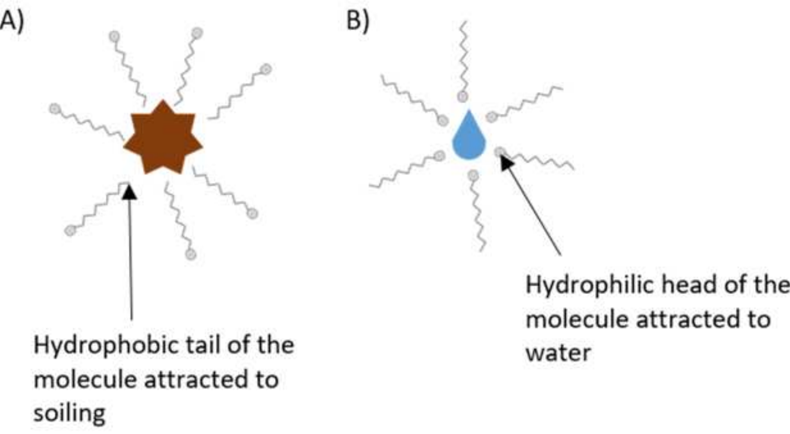


Figure 4.4 A) Soil particle with detergent molecules attracted to the particle with hydrophobic tail and B) water molecule with hydrophilic head of detergent attracted to water molecule.

The review identified N=18 studies assessing detergents. However, N=8 studies warned of the potential risk of increasing bacterial load during cleaning, or transfer to subsequent surfaces, likely from contamination of the detergent [4, 343, 345-350]. As detergents have no antimicrobial activity, they can readily become contaminated. This importance must be expressed during cleaning training, and is of even more importance when low level cleaning is undertaken with detergent alone (such as in general ward areas) with no follow-up of disinfectants. Detergents can be a single product, or contained as part of the formulation within a disinfectant. Disinfectants containing a detergent are advantageous to allow breakdown of organic matter and debris and disinfection all in one, though this single-step cleaning method increases the risk of missed areas, which may be caught with a traditional two step cleaning method.

Different cleaning agents are more effective against certain organisms, have varying compatibility with different surface materials, and require a range of personal protective equipment (PPE) or training for use. Additionally, the efficacy information provided by the manufacturer may not give a true insight into how well a cleaning agent may work for clinical surfaces. Cleaning agents are tested under different conditions using various protocols following different guidelines as per the target accreditation. There are two European standards for suspension testing of clinical disinfectants against bacteria (EN 13727) fungi (EN 13624) or mycobacteria (EN 14348). N= 169 studies were excluded from the review as they tested organisms in suspension only, and did not reflect performance on surfaces. N= 7 studies were

included that had both suspension and surfaces tested and the data were not included for consideration within this thesis. None of these studies had subsequent testing within a real clinical environment. Carrier testing, also known as 'phase 2' testing, challenges the cleaning agent against bacteria (EN 14561) fungi and yeast (EN 14562) or mycobacteria (14563) under a range of conditions related to real environments, such as the presence of soiling or a range of temperatures. Some cleaning agents are rigorously tested, with both carrier and suspension tests. Suspension testing, or testing pathogens in planktonic form, will give a 'best case' result, allowing the cleaning agent to work at its highest capacity. Carrier testing is crucial to gain insight into how well a cleaning agent will work on real surfaces. The addition of soil challenge testing can determine if an agent is deactivated in the presence of organic soiling, and to what extent this cleaning agent can still perform.

Clinical surfaces are often contaminated with soil, therefore a cleaning agent needs to be able to tackle this effectively. As explored from the literature review, many common cleaning agents have reduced kill capacity in the presence of soil, such as QUATS [351-354]. There are two proposed ways in which this soiling interferes with the disinfectants; either by the disinfectant being adsorbed by the soil and inactivated as a result, or reacting with the soil and becoming inactivated [355]. Therefore, the presence of soiling plays such an important role in cleaning agent efficacy, and cleaning agents must be tested in a way that is reflective of this. The review revealed how differently cleaning agents were tested. A large proportion of studies (N= 169, 25.4% of total assessed at full text stage) were excluded as they tested their cleaning agents with planktonic testing only. This represents a large

number of studies providing data inappropriate for evaluating how a cleaning agent will perform on surfaces in a clinical environment. Some of the literature assessed their agents with carrier testing and a soil challenge, though this level of testing is not standardised throughout the literature.

However, just choosing a cleaning agent that performs well in both carrier and suspension testing does not automatically make it the most suitable. Some of the more powerful cleaning agents, or agents used at lower dilution, can become less compatible with the user. Aldehydes, including formaldehyde, glutaraldehyde, ortho-phthalaldehyde and Dialdehyde are high-level disinfectants, which work well against bacteria, viruses, fungi spores and parasites in both liquid and gas states [356]. They also have the added benefit of residual activity on surfaces, prolonging their efficacy. However, they make a poor choice for general surface cleaning due to their toxicity and ability to degrade and stain some surface materials, as the literature consistently reported and warned of these limitations [356].

The wider concern of antimicrobial resistance should also be considered when selecting a cleaning agent. Some organisms have inherent (intrinsic) resistance to certain cleaning agents, as shown in figure 4.4.

Prions (CJD, BSE)
Coccidia (*Cryptosporidium* spp)
Bacterial endospores (*Bacillus* spp. *Clostridium difficile*)
Mycobacteria (*Mycobacterium tuberculosis, avium, terrae*)
Cysts (*Giardia*, *Taenia* spp)
Small non-enveloped viruses (Poliovirus)
Trophozoites (*Acanthamoeba* spp)
Gram-negative bacteria
Burkholderia cepacia
Providencia stuartii and Proteus spp.
Pseudomonas aeruginosa
Klebsiella pneumoniae
Acinetobacter baumannii
Escherichia coli
Fungi (including fungal spores) (*Aspergillus* spp, *Candida* spp)
Large non-enveloped viruses (Adenovirus)
Gram-positive bacteria (*Staphylococcus* spp, *Enterococcus* spp)
Large lipid enveloped viruses (HIV, HBV)

Figure 4.5 Classification of organisms according to resistance to disinfectants, as taken from Bock 2019.

Other organisms also have acquired the ability to adapt to disinfectants in various ways, such as modification of their membrane, upregulation of efflux pumps, or increase their propensity to form biofilms in response to biocides [357]. Careful selection, use and rotation of cleaning agents must be applied to reduce the prevalence of disinfectant-resistant organisms. As shown from tables 4.2-4.4, some organisms have the potential for developing resistance mechanisms to certain cleaning agents. This potential for previously sensitive pathogens to develop resistance to current disinfectants represents a real concern for the future of cleaning [357]. Currently, there is no known resistance mechanism to chlorine or

hydrogen peroxide, or physical cleaning agents as these work by physical removal. While chlorine is a powerful cleaning agent and stock rotation is not needed as there are currently no known resistance mechanisms, complete reliance on chlorine-based cleaning agents without rotation with other agents such as detergents will mean surfaces are continuously exposed to a known corrosive agent, and will reduce the lifespan of certain surfaces, such as plastic and stainless steel [358-360]. Therefore, the practicality of stock rotation must be considered in relation to the cost of more frequent repair and replacement of corroded or damaged surfaces, which may promote bacterial attachment (see section 1.4).

The literature revealed several studies in which reduced susceptibility to cleaning agents has been recorded [361] though to what extent this may impact clinical practice is currently unknown. Some studies have also reported that there is no difference in susceptibilities to cleaning agents between antibiotic resistant and antibiotic-susceptible pathogens [361].

Cleaning agent efficacy, frequency of cleaning, training quality and staff competency have an impact on how effective a cleaning protocol will be. For working with cleaning teams and showing them the effect of their cleaning, visual UV marker spots or the use of ATP devices has proven effective, while standard microbiological sampling may have little meaning to cleaning personnel. Ensuring good cleaning compliance is a key component in effective cleaning, as a cleaning agent will only be effective if used correctly.

4.7 CONCLUSION

Cleaning is an important intervention to prevent HCAI and is a critical component for IPC. However, cleaning protocols, guidelines and training is not standardised between hospitals, and cleaning varies both within UK hospitals and around the world. Additionally, there is a serious lack of evidence-based cleaning guidelines. The review of the literature revealed there are many options for surface cleaning in clinical environments, yet without the evidence, it can be impossible for IPC staff to determine what to use to clean their environment. The evidence was reviewed, and suggestions can be made for clinical cleaning, though more work and evidence is required.

Cleaning can be undertaken with liquid agents applied with a cloth or mop, commercially available packaged wipes, or with novel cleaning adjunct systems such as HPV or UV systems. Regardless of the agents chosen, physical removal of dirt with a detergent should always occur first, followed by a disinfection process if required, as disinfectants cannot work when challenged with heavy surface soiling. The cleaning agent chosen for each cleaning task has its own advantages and limitations; therefore careful selection is important to ensure cleaning is undertaken as effectively as possible. A modern world is revealing new and exciting technologies to undertake hospital surface cleaning, often being favoured and promoted as powerful and time-saving cleaning agents, such as UVC and HPV robots, though these systems are not without their limitations, and are not a replacement for physical surface cleaning.

Chapter 5 CLEANING WITH SURFACE WIPES

5.1 INTRODUCTION

There are many possible options for undertaking surface cleaning for smaller surfaces or for spot-cleaning. Using cloths and a spray disinfectant, or ready to use (RTU) commercially available wipes pre-impregnated with a specific concentration of cleaning agent, represent an easy and economical method of cleaning surfaces. Data suggests that these ready to use wipes are rising in popularity and are being used more than ever in healthcare settings [362]. There are a multitude of pre-packaged surface wipes available for cleaning clinical surfaces, with a range of uses and compatibilities [362, 363]. As with all cleaning agents, they must be used properly in order to be most effective at cleaning and removing soiling and organisms from a surface. Improper use can compromise their cleaning ability [200].

Ready to use wipes have several advantages. They require no preparation or dilution prior to use, with the exception of some wipes that require wetting for activation, such as the GAMA healthcare peracetic-acid based sporicidal wipes. This reduces the risk of using too little cleaning agent and diluting the active ingredient too much, which is a potential concern with liquid cleaning agents that require preparation, being diluted by guesswork or over-diluted. There is no risk of contaminating the cleaning agent during use, preparation or storage, such as using contaminated water, or preparing in a contaminated bucket. Additionally, as they are disposable, any organisms are retained within the wipe and discarded safely. Reusable cloths must be used carefully as they carry the risk of this transmission across surfaces if the cloth becomes soiled or is used for multiple surfaces.

Liquid cleaning agents are useful for cleaning larger surfaces, such as walls and floors. They are cheap and economical and can produce large volumes of cleaning agent, which are required to clean large surfaces within a ward. Liquid cleaning agents are applied with cloths or mops, and the most common liquid cleaning agents used in the clinical environment are bleach (sodium hypochlorite) NaDCC (sodium dichloroisocyanurate) and Tristel-brand (chlorine dioxide). These come in tablet or sachet forms, in which a specific number of tablets are sachets are added to a certain volume of water to produce an agent at a useable concentration.

As with all cleaning agents, assessment standards vary. Cleaning agents can be evaluated on surfaces in a carrier test, or planktonic testing. An ideal test standard would show the performance of the wipe or cleaning agent under both carrier and planktonic testing, with additional testing under conditions replicating surface soil, mimicking the environment a wipe will be challenged with within the clinical setting. Several studies have been undertaken assessing wipes with different methods using a range of bacteria, spore formers or viruses, inoculated onto a range of surface materials [362]. It was found that the compatibility of the disinfectant and the wipe material must be considered, and that more information is needed on how to use the RTU wipes.

Only a few studies have assessed wipes within the clinical environment. Testing under these conditions are likely to show the true performance of surface wipes, as they are challenged with a wide range of conditions such as soiling, variation of surface types and materials, potential presence of heavy bacterial loading and established biofilm presence. However, comparison between clinical-

based tests and laboratory-based tests is difficult and challenging due to the discrepancies in methods used [362, 363]. A review by Boyce (2021) compiled the current data for wipes used in healthcare settings, and reiterated the need for further research on different types of wipes [362]. This study was limited in its search until 2019, so newer studies were not included. Within the review, only 2 studies assessed wipe performance on ceramic tiling, which forms in important part of the hospital surface environment, and tiled bathroom and sink areas have been linked to significant outbreak (section 1.3.4). Within these 2 studies, *P. aeruginosa* was not tested, which is of specific concern as this pathogen has been linked to outbreaks from bathrooms and sink areas [41-43]. The wipes specific to the setting of this thesis, a UK pediatric hospital, were not tested in any of these studies. While a bleach-based Clorox branded was tested with the review, it has a different formulation than the Clinell-Clorox branded wipes used in UK facilities. Reproducibility across multiple studies can give confidence of data, therefore relying on one or two publications does not mean we can be entirely convinced of the efficacy of a wipe. Multiple studies undertaking the same methodology reproducing the same or similar results bridging all possible gaps (such as different surfaces, wipe and cleaning agent chemistries, different pathogens and different strains of the same organism, variation of temperature and humidity and other testing conditions) is needed as evidence to allow sophisticated meta-analyses of the data, which is the gold standard of providing an evidence-based review to add significant value to any study area [364]. But for a meta-analysis, significant numbers of high-quality studies

are required and this thesis makes that contribution to start building a more concrete picture of wipe efficacy.

Therefore, knowing exactly how effective a wipe is, how rigorously it has been tested and how well it might perform in a real clinical environment can be a challenging question. With the availability of many types of RTU surface wipes and liquid cleaning agents, it can be difficult for healthcare professionals to make informed and confident decisions on which cleaning agents will work best in their hospitals.

5.2 RESEARCH AIMS

The aims of this chapter were to explore the effectiveness of surface wipes used within the clinical environment. The following aims were considered and explored;

1. How do different cleaning agents work when challenged with clinically significant pathogens? An assessment of commercially available wipes and liquid cleaning agents, to explore efficacies against different clinically significant pathogens.
2. Discuss what these results might mean for the efficacy of the wipes and their use in clinical practise.

5.3 METHODS

The following methods were designed, as there are no current industry standards for in-use wipe testing.

5.3.1 MATERIALS

White ceramic tiles were used as representative non-porous surfaces, as in section 3.3.2. For pre-packed wipe testing, three commercially available RTU wipes for clinical surface cleaning were tested. Gauze provided a control without an active ingredient. For liquid cleaning agent testing, three agents were tested. These materials were chosen as they are the cleaning agents used within the setting for all practical work undertaken within this thesis, a UK-based NHS pediatric hospital. All data based on cleaning training and cleaning efficacy and surrogate transmission will be related to the following agents used within the pediatric setting.

Table 5.1 Details for surface wipes and liquid cleaning agents used within this study, with manufacturer and formulation details.

	Common name	Chemistry	Brand	Concentration	Manufacturer website
Ready to use wipes	Clinell Universal	Undisclosed QUAT-based formula	Clinell	<0.5% benzalkonium chloride	https://www.gamahealthcare.com/products/universal-range
	Alcohol wipe	Isopropyl alcohol	PDI	70%	https://pdihc.com/global/products/environment-of-care/sani-cloth-70/
	Clorox wipe	Sodium hypochlorite (NaClO)	Clinell/ Clorox	5200ppm available chlorine <1%	https://www.gamahealthcare.com/products/clorox-range
	Cloth (gauze)	n/a	NHS supplier	n/a	n/a
Liquid Cleaning Agents	Bleach	Sodium hypochlorite (NaClO)	Sigma-Aldritch	1:100 dilution to produce 1000ppm, 10-15% available chlorine	https://www.sigmaaldrich.com/catalog/product/mm/105614?lang=en®ion=GB
	Tristel	Chlorine Dioxide (ClO_2)	Tristel	>20ppm (1 sachet per 5 litres) <1% available chlorine dioxide	https://www.tristel.com/uk/cache-products/fuse
	NaDCC	Sodium dichloroisocyanurate	Guest Medical	1000ppm (1 tablet per 1 litre) 10-30% available troclosene sodium	https://guest-medical.co.uk/chlor-clean/

5.3.2 SURFACE INOCULATIONS

White ceramic tiles were prepared as previously in section 3.3.2. Cultures of *S. aureus* and *K. pneumoniae* were prepared as in section 3.3.1. Tiles were divided into 4 sections to create 4 replicates for sampling. The method series is shown in figure 5.1 below. A total of 250µl of each organism suspension was aliquoted onto each tile quarter in 5x50µl droplets, equivalent to an approximate concentration of 10^8. The drops were allowed to dry for 2 hours prior to testing.

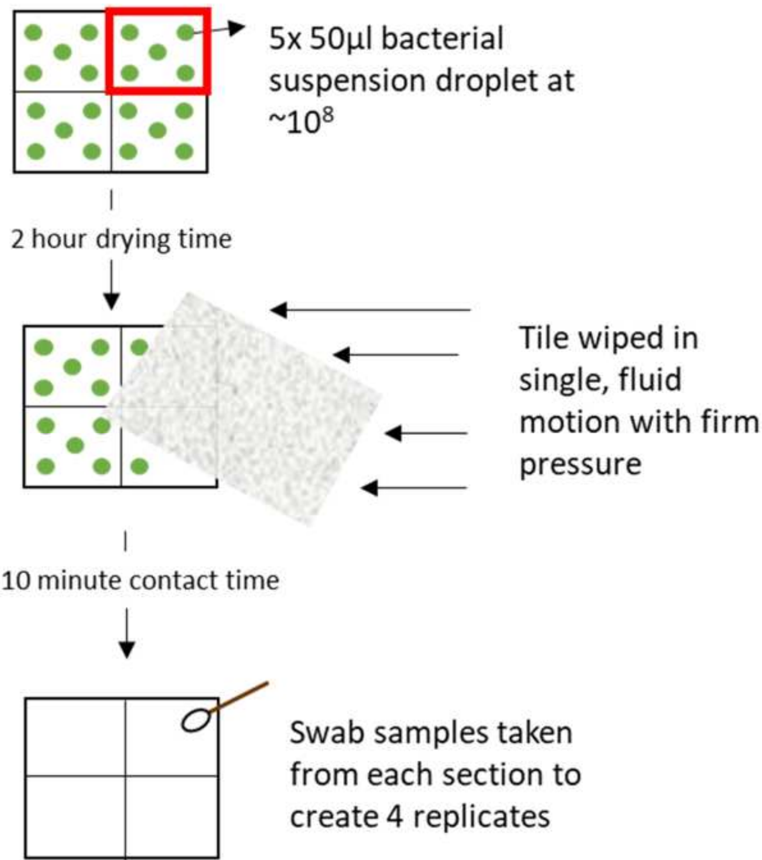


Figure 5.1 Graphical Sampling methodology for surface inoculations

5.3.3 SURFACE WIPE CLEANING METHOD

Once the inoculum had dried for 2 hours, and assessed visually to ensure drops were dry, tiles were ready to be challenged with the wipes.

For RTU wipe testing, each tile was cleaned with a one of the test wipes (Universal Clinell wipe, a Clorox-Clinell wipe, an alcohol wipe or gauze) in a single, firm, fluid motion. The wipe was then disposed of as per local instructions. To replicate the conditions of the other RTU wipes, the gauze was wetted with 15ml sterile PBS prior to use.

For liquid cleaning agent testing, a J-cloth (to replicate that used in the hospital) soaked in bleach solution, chlorine dioxide (Tristel) or NaDCC (see table 5.1 for active ingredient concentrations) in a single, firm fluid motion. Soaking was undertaken following the GOSH standard cleaning procedure, where the cloths were soaked in a bucket of the cleaning agent for 2 minutes.

For tests requiring a secondary wipe, second wipes were taken using the same method immediately following discard of the first wipe. Once wiping was complete, a contact time of 10 minutes was allowed for the RTU wipes, or contact times of 10, 60 and 120 minutes for the liquid cleaning agents was allowed to expose the tile to the cleaning agent deposited from the wipe. Once the contact times were complete samples were taken using cotton swabs, as in section 3.3.4.2. For the 60 and 120 minutes, 'contact time' refers to residual activity, as once dried the agent was not reapplied.

Immediately after sampling and vortexing for 60 seconds, serial dilutions were prepared. A neutralisation step was not used as the samples were processed and diluted immediately; therefore any residual cleaning agent was diluted. The dilutions were plated onto blood agar plates, spread in a figure-eight motion using sterile spreaders, and incubated at 37°C for 24-48 hours. Following incubation, colonies were counted. Figure 5.2 shows the graphical method process.

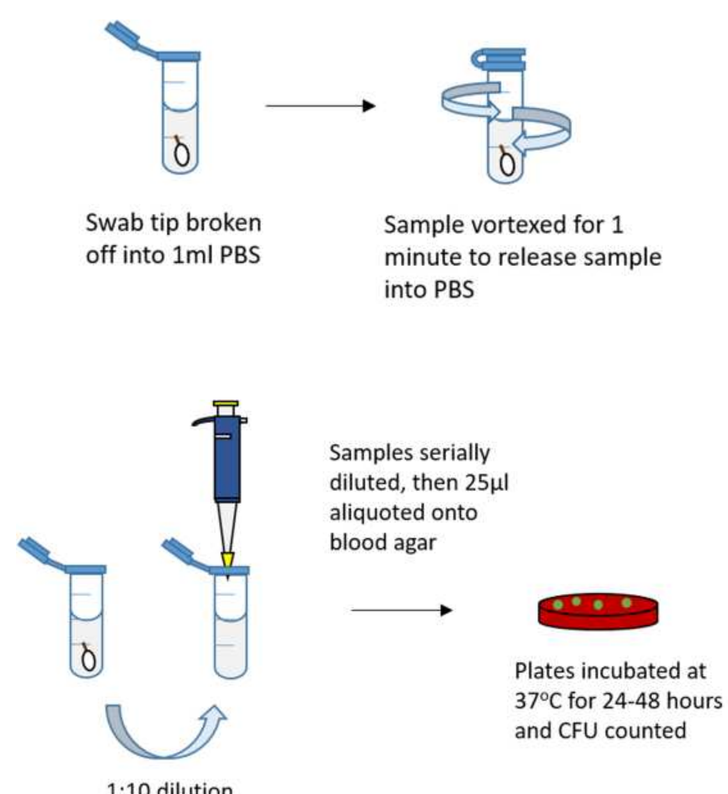


Figure 5.2 Graphical Sampling methodology for swab processing.

5.3.4 WIPE LIQUID CONTENT TESTING

To assess liquid content, the first two wipes from each wipe pack were discarded. Four additional wipes were removed from each container of the green Clinell, alcohol and Clorox wipes. These wipes were immediately weighed, allowed

to dry completely, then re-weighed to assess their moisture content. For the gauze wipes, they were dampened with 15ml of PBS as described in section 5.3.3.

5.3.5 WIPE MANUFACTURER INSTRUCTION ASSESSMENT

An assessment of 11 global brands providing RTU wipes for the healthcare industry were assessed for the type and quality of information they provide. Wipes were scored on the quality and amount of information provided by the manufacturer. Information provided from the manufacturer was assessed from instructions on the wipe packaging, leaflets and product data downloadable from their websites, and any efficacy data provided. The criteria chosen represent the possible available information a wipe manufacturer could provide to their user, using the well-known manufacturer GAMA healthcare and discussions with IPC professionals as the gold standard. These represent the components a user would need to know in order to use the wipes effectively, under the assumption that the user were not receiving regular and robust cleaning refresher training. The easy accessibility of such information can mitigate certain issues cleaners find when needing additional help when using wipes, such as forgetting steps or feeling uncomfortable with asking for help from a coworker or line manager through fear of being reprimanded, or to try and prevent the risk of newer cleaning or healthcare staff only learning through ‘hand me down’ information from peers which gets diluted and bad habits are passed along outside of formal, evidence-based training. With these criteria in mind, wipes were scored based on the following;

- **Is there a visual training protocol?** Either in the form of a diagram, photographs, or instructional videos showing how to use the wipes.

- **Does it show or mention donning and doffing of PPE?** Using gloves and apron, or other appropriate PPE.
- **Removing prior visible soiling?** Disinfection cannot occur in the presence of organic matter and other soiling. All visible soil must be removed before disinfection of the surface can occur.
- **Unfolding wipe fully before use?** Unfolding the wipe properly is often overlooked as an important part of the cleaning process. This can be shown in the visual training photos or as text.
- **Appropriate wiping technique/ motion?** Appropriate wiping technique allows efficient removal of bioburden. Poor technique can lead to the spread of organisms. It is important to ensure all surface comes in contact with the wipe, with enough overlap, in an 's' motion.
- **Pressure application?** Pressure is a vital step in removing bioburden from a surface, particularly if organisms have formed a biofilm. Using too little pressure will not allow removal of organisms and other surface contaminants.
- **Contact time?** Contact time is the time the surface remains saturated with the active ingredient and remains wet for that time. Incorrect contact times can lead to poor disinfection of a surfaces and infection transmission. It is important to note that different organisms have different contact times, and different wipes kill organisms at different times. It is important for this

information to be freely available so the technician can ensure appropriate contact times are adhered to, allowing the best disinfection results.

- **Ensuring the surface is dry?** Once contact time is complete, it is important to allow the surface to dry before use or disturbance. Some chemicals have a residual effect on the surface even when dry.
- **Disposal instructions?** Can the wipes be disposed of in biological waste bins, or regular waste?
- **Coverage per wipe?** Surface area per wipe is not finite; it is important to ensure a wipe is not exhausted or too heavily contaminated. Using a wipe over a too-large surface area will lead to the spreading of organisms around the surface and infection spread.
- **Closing wipe pack securely?** Closing the pack is important and sometimes a reminder can be given on the packs or tubes, as dry wipes are ineffective and contact time will not be reached.
- **Training video availability?** Some companies provide training videos, showing wipe technique and other features of their product. This can be useful training information for technicians and reminders on how best to use the wipe.

5.3.6 STATISTICAL ANALYSIS

Data (CFU recovered) were assessed for normality and distribution within IBM SPSS. The data violated ANOVA assumptions; therefore Kruskal-Wallis test was performed. To assess wipe liquid content, paired samples t-test was performed.

5.4 RESULTS

5.4.1 WIPE CLEANING RESULTS

The results showed that the most effective wipes were the green Clinell and Clinell-Clorox wipes. Clinell Universal and Clinell-Clorox wipes both achieved mean log reductions of 8.13 for *S. aureus* and 8.02 for *K. pneumoniae*, total kill, with a single wipe and 10 minute contact time (table 5.3). As total reduction (within LOD, ≤4.00E+01 CFU) was achieved, a second wipe did not improve efficacy for Green Clinell and Clorox wipes.

For alcohol wipes, mean log reductions of 2.22 and 3.73 $\log_{10}$ were achieved for *S. aureus* and *K. pneumoniae* respectively with a single wipe. A second wipe improved removal for *S. aureus* (improving to 4.30) and for *K. pneumoniae*, there was no improvement, at 3.32 (table 5.3).

For gauze, log reductions of 4.37 and 3.20 were achieved with a single wipe, for *S. aureus* and *K. pneumoniae* respectively (table 5.3). A second wipe improved removal of both organisms, improving to 5.04 for *S. aureus* and 4.32 for *K. pneumoniae*.

There was no statistical significance between log kill and wipe type (P= .080187). The two Clinell-branded (Clinell Universal and Clinell-Clorox) wipes achieved and surpassed the required >5 log reduction required for clinical surface wipes [194] in a single wipe for both organisms. Alcohol wipes did not meet this criteria within these testing conditions, for either organism, using two wipes. Wetted

gauze achieved this standard for *S. aureus* only with two wipes (table 5.3). There was a statistically significant difference in recoveries of the different organisms ($P < .001$). Gauze wipes outperforming alcohol wipes were addressed further in section 4.4.2.1, assessing if the liquid content or 'wetness' of the wipe was a factor contributing to the efficacy.

Table 5.2 Wipe efficacy testing with single or multiple wipes for *S. aureus* and *K. pneumoniae* on ceramic surfaces (each result is the mean of N= 4 replicates, also see figure 5.4)

	Wipe efficacy for *S. aureus*				
	Wipe type	Control / CFU/ml	Test/ CFU/ml (SD)	log kill (SD)	% kill
	Inoculum	1.4E+08			
	2 hour viability	1.4E+08	6.50E+05 (1.00E+06)	2.32 (1.11E-01)	99.523
Single wipe	Clinell	1.4E+08	<4.00E+01 CFU	8.13	100
	Clorox	1.4E+08	<4.00E+01 CFU	8.13	100
	Alcohol	1.4E+08	8.E+05 (1.04E+06)	2.22 (1.70E-1)	99.397
	Gauze	1.4E+08	6.E+03 (5.29E+02)	4.37 (2.73E+00)	99.996
Two wipes	Clinell	1.4E+08	<4.00E+01 CFU	8.13	100
	Clorox	1.4E+08	<4.00E+01 CFU	8.13	100
	Alcohol	1.4E+08	6.88E+03 (3.38E+04)	4.30 (1.78E+00)	99.995
	Gauze	1.4E+08	1.25E+03 (5.29E+02)	5.04 (2.73E+00)	99.999
	Wipe efficacy for *K. pneumoniae*				
	Wipe type	Control / CFU/ ml	Test/ CFU/ml (SD)	log kill (SD)	% kill
	Inoculum	1.1E+08			
	2 hour viability	1.1E+08	1.050E+07 (3.79E+06)	1.00 (3.79E-01)	90.000
Single wipe	Clinell	1.1E+08	<3.30E+01 CFU	8.02	100
	Clorox	1.1E+08	<3.30E+01 CFU	8.02	100
	Alcohol	1.1E+08	1.94E+04 (7.12E+04)	3.73 (1.88E+00)	99.982
	Gauze	1.1E+08	6.59E+04 (1.02E+05)	3.20 (1.75E+00)	99.937
Two wipes	Clinell	1.1E+08	<3.30E+01 CFU	8.02	100
	Clorox	1.1E+08	<3.30E+01 CFU	8.02	100
	Alcohol	1.1E+08	5.06E+04 (3.09E+05)	3.32 (1.60E+00)	99.952
	Gauze	1.1E+08	5.06E+03 (1.21E+04)	4.32 (2.52E+00)	99.995

SD = standard deviation. Limit of detection (LOD) for *S. aureus* (4.00E+01) and *K. pneumoniae* (3.30E+01)

5.4.2 WIPE WETNESS AND PERFORMANCE

There was found to be a significant difference between the liquid content of the gaze and alcohol wipes (P< 0.001) and the two Clinell-branded wipes (P< 0.01). During the experiment, it was found that the most effective wipes, green Clinell and bleach wipes, were found to be wetter and produce more liquid during use than the alcohol wipe. The least effective wipes, the alcohol wipes, were found to be drier. For *S. aureus*, the wetted gauze wipes outperformed the alcohol wipes. As the gauze wipes were used as a control, with no active ingredient, it was determined that this was either a difference in mechanical removal due to the fabric weave, or by the amount of liquid contained within the wipe.

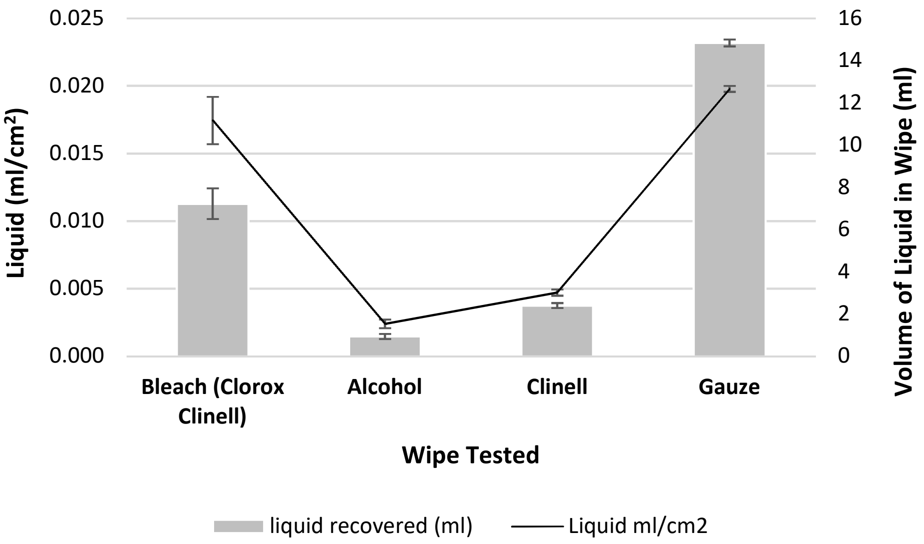


Figure 5.3 Volume of Liquid Contained (mean) within Different Types of Surface Wipes (n= 4)

The results showed the wipes that were determined to be wetter during use, green Clinell (universal) and Clinell/Clorox (bleach), were more effective than the drier alcohol wipes. The gauze wipes were the wettest, as they were wet with 15ml PBS manually prior to use, yet did not have the most log reduction.

5.4.3 LIQUID CLEANING RESULTS

When using traditional liquid cleaning agents, the results showed that different contact times had an important impact on log kill.

Table 5.3 Mean Log kill achieved against *S. aureus* using bleach, NaDCC or chlorine dioxide liquid cleaning agents for 10, 60 or 120 minute contact times (also see figure 5.4).

	Exposure (min)	Control / CFU/ ml	Test/ CFU/ ml	log kill	% kill
Bleach (1000ppm, 10-15% available chlorine)	10	3.50E+07	2.11E+03	4.2	99.994
	60	4.00E+07	<4.00E+01 CFU	9.6	100
	120	3.50E+07	<4.00E+01 CFU	8.5	100
NaDCC (1000ppm 10-30% available troclosene sodium)	10	3.50E+07	9.25E+02	4.6	99.997
	60	4.00E+07	<4.00E+01 CFU	9.6	100
	120	3.50E+07	<4.00E+01 CFU	8.5	100
Chlorine Dioxide (>20ppm <1% available chlorine dioxide)	10	3.50E+07	5.85E+03	3.8	99.983
	60	4.00E+07	1.86E+03	4.3	99.995
	120	3.50E+07	1.25E+01	6.4	100

Table 5.4 Log kill achieved against *K. pneumoniae* using bleach, NaDCC or chlorine dioxide liquid cleaning agents for 10, 60 or 120 minute contact times (also see figure 5.4).

	Exposure (min)	**Control / CFU/ ml**	**Test/ CFU/ ml**	**log kill**	**% kill**
Bleach (1000ppm, 10-15% available chlorine)	10	5.00E+07	1.76E+03	4.5	99.996
	60	5.00E+07	<3.30E+01 CFU	8.7	100
	120	5.00E+07	<3.30E+01 CFU	8.7	100
NaDCC (1000ppm 10-30% available troclosene sodium)	10	5.00E+07	5.63E+02	4.9	99.999
	60	5.00E+07	<3.30E+01 CFU	8.7	100
	120	5.00E+07	<3.30E+01 CFU	8.7	100
Chlorine Dioxide (>20ppm <1% available chlorine dioxide)	10	5.00E+07	2.26E+03	4.3	99.995
	60	5.00E+07	<3.30E+01 CFU	8.7	100
	120	5.00E+07	<3.30E+01 CFU	8.7	100

For both organisms, a 60 minute contact time with bleach and NaDCC achieved total kill (within LOD). A contact time of 120 minutes did not, therefore, lead to improved kill. For ClO_2, to achieve complete kill of *S. aureus*, a 120 minute contact time was required.

5.4.4 READY TO USE WIPES VERSUS LIQUID CLEANING AGENTS

When comparing the RTU wipes against the cloths soaked in the liquid cleaning agents, against *S. aureus* and *K. pneumoniae,* the results showed that for the liquid agents exposure time played an important role. For the liquid cleaning agents given a 10-minute contact time, both Clinell wipes performed better. At a 10-minute contact time, the liquid agents achieved reductions of 3.78-4.95 log_{10}, whereas the RTU wipes achieved 2.22-8.13 log_{10} (figure 5.4). At a ≤60-minute contact time, bleach and NaDCC performed comparably to the RTU wipes. Extending to 120 minutes did not improve efficacy for bleach or NaDCC, but allowed ClO_2 to improve from 3.78 to 6.45 log_{10} for *S. aureus*, and from 4.34-8.70 log_{10} for *K. pneumoniae*. Overall, the RTU wipes worked better against *S. aureus* than liquid agents with short (10 minutes), contact times, whereas the liquid agents worked better for *K. pneumoniae* achieving best reduction up to 8.7 log_{10} with 60 minute contact times, whereas wipe methods for K. pneumoniae only achieved up to 8.13 log_{10} reduction with two consecutive wipes. For both *K. pneumoniae* and *S. aureus*, the alcohol wipes (PDI brand, tested within the conditions of this single study) failed to meet the criteria of ≥ 5log_{10} reduction required to perform as an effective disinfectant.

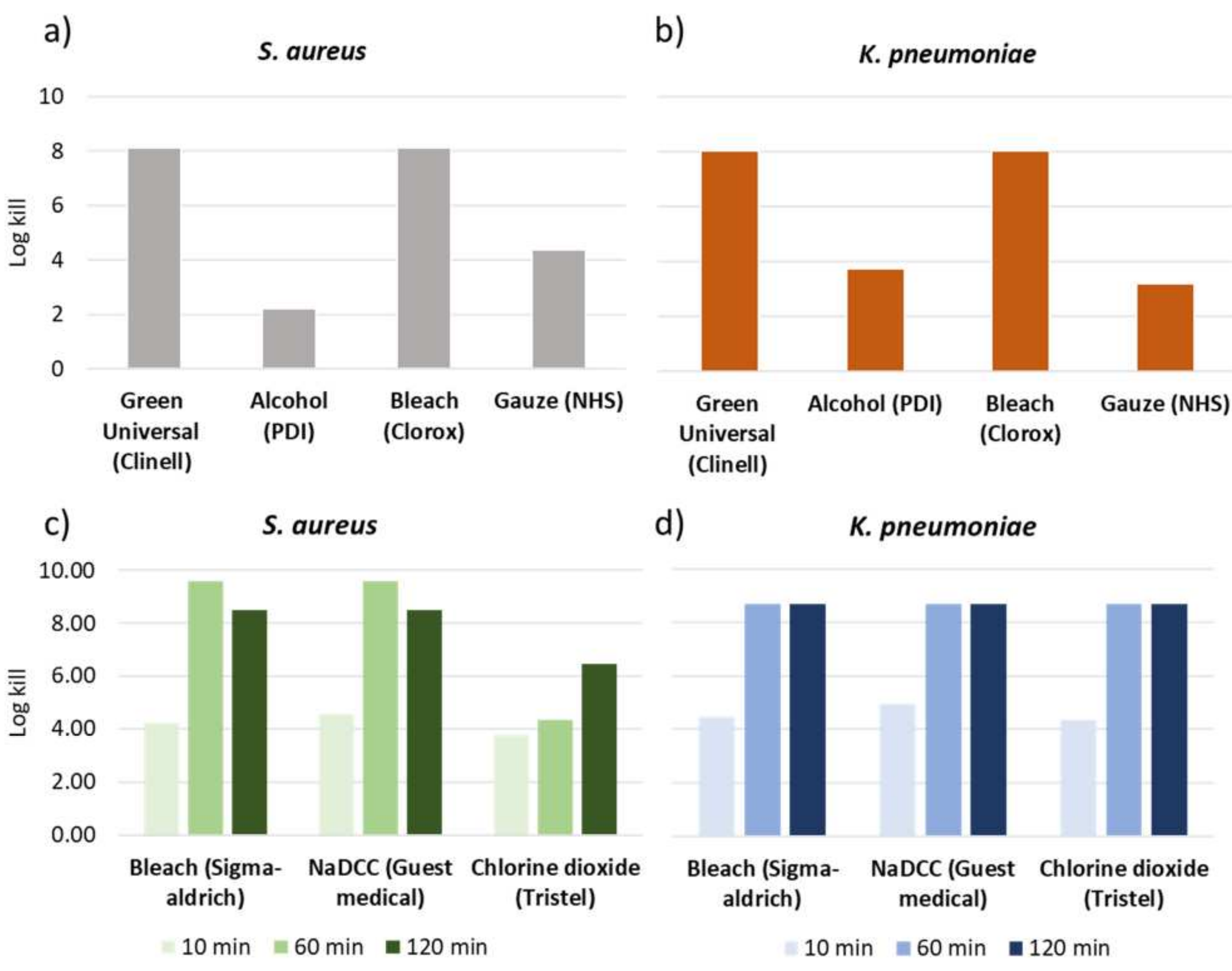


Figure 5.4 Mean Log reductions of *S. aureus* and *K. pneumoniae* on ceramic tiles cleaned with RTU commercial wipes (a and b) or cloths soaked in liquid cleaning agent with contact times of 10, 60 or 120 minutes (c and d).

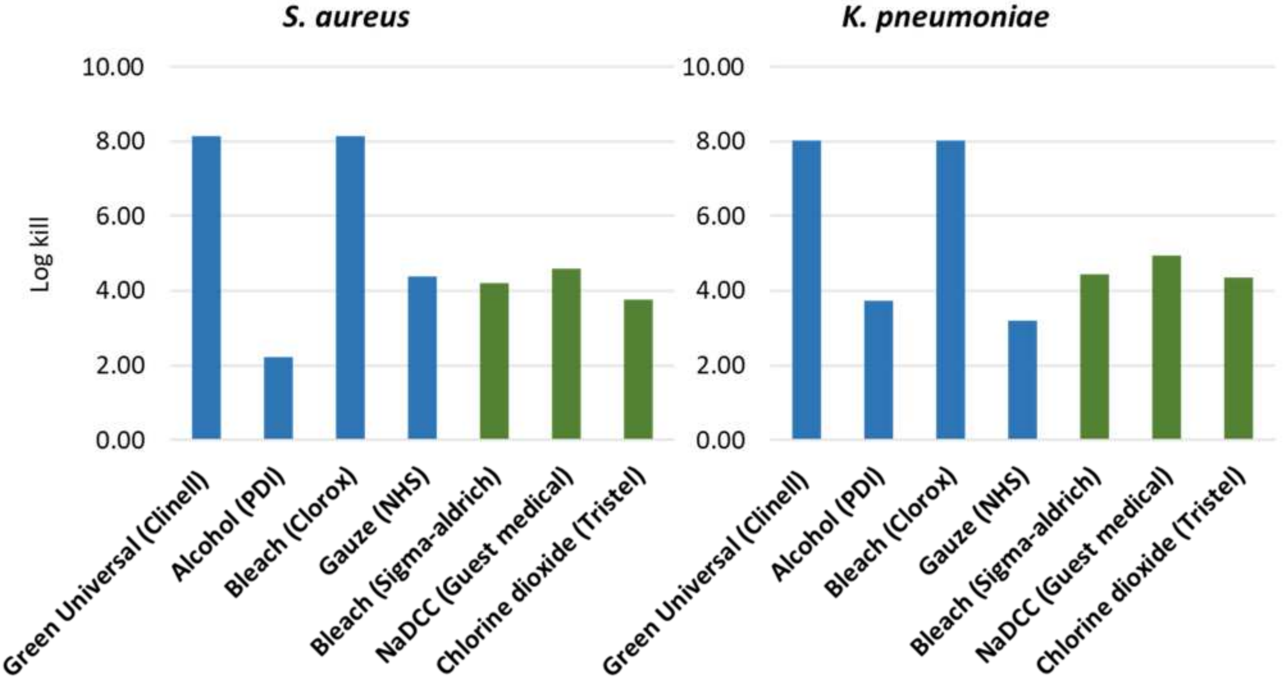


Figure 5.5 Comparison of mean log reductions of *S. aureus* and *K. pneumoniae* on ceramic tiles cleaned with either RTU wipes (blue bars) or liquid cleaning agents (green bars) both with a 10-minute contact time.

5.4.5 Manufactures' instructions assessment results

The manufacturer instructions available on how to use different RTU wipes varied considerably. Some manufacturers provide clear, precise instructions both on their packaging and available to download as posters and information packages from their websites, while others provide limited or no instruction. For users, especially when re-training of cleaning is lacking (chapter 7), instructions on use on the package can provide essential information to allow proper and effective cleaning. All wipes (N= 19 individual wipes) were designed towards the healthcare environment, except the Texwipe brand that catered towards industry cleanroom cleaning

Table 5.5 Quality of information provided by wipe manufacturers (bold denotes tested wipes, each * awarded represents information available in each criteria)

Manufacturer	Wipe Name	Main Active Ingredient(s)	Quality of Information
Metrex	Caviwipes	Propan-2-ol, isopropyl alcohol, isopropanol 17.20%, 2-Butoxyethanol, ethylene glycol monobutyl ether, butyl cellosolve 1-5%, Benzethonium Chloride 0.28%	*
PDI	Sani-Cloth AF3	Quaternary ammonium compounds, C12- 18-alkyl [(ethylphenyl) methyl] dimethyl, chlorides 0.14%, Benzyl-C12-18-alkyldimethyl ammonium chlorides 0.14%	**
	Super Sani-cloth	Isopropyl alcohol 55.5%, uaternary ammonium compounds, C12-18-alkyl [(ethylphenyl) methyl] dimethyl, chlorides 0.25%, n-Alkyl Dimethyl Benzyl Ammonium Chloride -.25%	
	Sani-cloth Bleach	Sodium hypochlorite 0.63%	
	Sani-cloth 70%	Propanol-2-ol 70%	
GAMA healthcare	**Clinell Universal**	Undisclosed QUAT formula, <0.5% benzalkonium chloride	****
	Sporicidal	Sodium Percarbonate 40-50%, Cirtric acid ≤15%	
	Spill	Sodium percarbonate ≤50%, Cirtric acid ≤0.5%	
	Detergent	Caprylyl/Capryl Glucoside ≤0.5%, Lauryl Polyglucose ≤0.3%, Butoxydiglycol ≤0.2%	
	Alcohol	Propan-2-ol 70%	

	Clorox	Sodium hypochlorite 0.74%, sodium hydroxide <0.25%	
PAL	Medipal	Didecyldimethylammonium chloride <1%, -(3-Aminopropyl)-Ndodecylpropane-1, 3-diamine <1%, Alpha.-Undecyl-omega hydroxypoly(oxy-1,2-ethanediyl) branched and linear <1%, 2-Aminoethanol <1%	*
USF	Unisepta	didecyldimethylammonium chloride 0.14g/100g, polyhexamethylene biguanide 0.09g/100g, and excipient	**
Contec	Sanotex	n/a provided as dry wipe to be compatible with all cleaners, cleaning agent of choice to be added to canister for use	*
Synergy Health	Azowipe	Propan-2-ol 70%	**
Texwipe	Vertex	Isopropyl alcohol 70%	***
Ecotech	Surface disinfectant wipes	Didecyldimethyl ammonium chloride 1-3%, isopropanol (Propan-2-ol) 1-10%	*
Vernacare	Tuffie	Polypropylene, <5% non ionic and amphoteric surfactants	*
Best Sanitizers INC	Alpet D2	Isopropyl alcohol 55-65%	*

None of the manufacturers provided the limit to the surface area their wipes can cover. Discrepancies were also found in which Metrex-branded wipes showed donning PPE in the information provided on the wipe pack however, in the visual training video, the wipes were handled without gloves. Unisepta provided visual instruction, however the wipe was not pressed flat against the surface. Only two companies stated their contact times on the packaging. Texwipe, a pharmaceutical wipe manufacturer, were the only company to provide information on cleaning from the cleanest part of the surface to the dirtiest, to prevent dragging contamination across a surface. They were also one of two manufacturers, alongside GAMA healthcare, to give details on overlapping while cleaning by 10-25% to ensure none of the surface is missed. This suggests, as with surface sampling device options and setting pass/fail criteria for sampling and establishing environmental monitoring programmes (chapter 2), much can be learned from the food and pharmaceutical industry than can be readily applied or adapted to healthcare settings.

5.5 DISCUSSION

This study sought to assess how well 3 RTU commercially available wipes performed when challenged with two clinically significant pathogens, on a representative non-porous surface, and how well liquid cleaning agents performed in comparison. The wipes varied in efficacy, depending on the type of wipe and organism. Additionally, it was revealed that the ‘wetness’ of the wipe played a role in how well the wipe performed. The Clinell branded wipes, green Universal and Bleach/Clorox wipes performed similarly, and achieved total reduction within the allowed 10-minute contact time.

It is important that the cleaning tools provided to healthcare professionals work quickly and effectively, as claimed by the manufacturer. Wipes that perform poorly due to variation in how the manufacturer chooses to test the wipes, local policy, manufacturer instruction and quality of cleaning training are all compounding factors that can lead to poor cleaning.

5.5.1 PERFORMANCE OF RTU WIPES AND LIQUID AGENTS

Depending on the type of active ingredient, contact time and target organism, all the cleaning agents performed differently. The wipes have the advantage of being pre-prepared and ready to use. With liquid cleaning agents, the additional step of dilution can introduce accidental over-dilution of a product, which will make it less effective as the minimum inhibitory concentration (MIC) is not being reached. The MIC is defined as the lowest concentration of an agent, in this instance a cleaning agent, to result in microbial death [365]. If incorrect preparation occurs, the cleaning agent may be used as a concentration below the MIC and will allow organisms to

survive following exposure to the cleaning agent. While some cleaning agents have no known mechanisms for resistance, such as chlorine, other cleaning agents are more susceptible. As discussed in section 1.3.6 in relation to antibiotics, adaptive resistance is a mutation in response to the environment to improve the chances of survival. Exposure to sub lethal concentrations of cleaning agents can allow this resistance and has been demonstrated in the literature, such as a study by Kampf (2018) demonstrating adaptation of a range of organisms to low level benzalkonium chloride surface cleaner [366, 367]. A maximum 4-fold increase in MIC was found for the 57 species of organism tested, and strong, adaptive changes in MIC were found for *Pantoea* spp., *Enterobacter* spp., *Staphylococcus saprophyticus* and *E. coli [366].* Additionally, using contaminated buckets or water may also reduce the efficacy of the cleaning agent, as clinically significant organisms have been recovered from disinfectant buckets [368] and detergent buckets [369]. Together, these factors can have disastrous consequences. A study by Boyce and Havill (2022) found that a bucket of disinfectant was used without emptying and drying the bucket between uses, leading to contamination of the disinfectant with high levels (9.3×10^4 CFU) of *Serratia marcescens* and *Achromobacter xylosoxidans,* two pathogens known to cause HCAI, which were not present on surfaces prior to disinfectant [370]. Sequencing of *S. marcescens* isolates identified genes (*sdeXY, sdeAB, smfY,* and a *sugE*-like gene) that to encode for efflux pumps associated with QUAT resistance [370].

Therefore, correct and careful in-house dilution and appropriate use of cleaning agents is critical, and incorrect compliance can cause spread of clinically

significant pathogens. As wipes are pre-prepared and single use, they represent a great alternative to mitigate this risk.

Another finding from these results highlight how Gram-negative and Gram-positive organisms survive on surfaces and resist desiccation. As previously discussed in section 1.3.5, Gram-negative organisms are more susceptible to environmental conditions as they do not have the thick peptidoglycan later than Gram-positives, such as *S. aureus,* have to protect them from the harsh environment. After drying on the tiles for 2 hours, in the absence of wiping, *S. aureus* had a 2.32 log_{10} reduction, while *K. pneumoniae* had a 1.00 log_{10} reduction. *S. aureus* is known for environmental survival and desiccation tolerance and has several genes that allow resistance to desiccation such as *ahpC*, *kata clpX* (controls osmotic and oxidative stress responses*),* sigB (controls 251 genes related to the stress response*) and yjbH* [284]. While *K. pneumoniae,* a Gram-negative organism does not have thick peptidoglycan that aids environmental survival, there are other factors that could explain this greater survival compared with *S. aureus.* This study was undertaken using ceramic tiles. Data from chapter 3 revealed that *K. pneumoniae* was more readily recovered from ceramic tiles compared with S. aureus, recoveries of 27.75% recovery against 18.42% recovery (P<.0001). *K. pneumoniae* and *S. aureus* are also shaped differently. *K. pneumoniae* are larger and rod shaped, while *S. aureus* are cocci and smaller. Section 3.5.2 explores the relationship between organism morphology and sampling device. Finally, while it may be expected that *S. aureus*, a Gram-positive, may resist desiccation more readily than *K. pneumoniae*, a Gram-negative, *K. pneumoniae* is known for its ability to persist and survive in the environment (section 1.3.2, 1.3.5)

and environmental survival of *K. pneumoniae* is very variable within the literature as shown in table 1.2 (reported between 2 hours and > 30 months) against *S. aureus* (reported between 2 days and 7 months) and within this study, the viability samples were taken after just 2 hours.

However, wipes cannot be used for all cleaning and are not suitable for large surfaces, or for walls and floors. Here, liquid cleaning agents must be used. Contact time for liquid cleaning agents was critical for greater log reductions. Particularly for ClO_2, in which a 120 minute contact time was needed to produce a >6 log_{10} reduction for *S. aureus*, and a ≤60 minute contact time for >8 log_{10} reduction for *K. pneumoniae*. For surfaces that can be cleaned with wipes, Clinell Universal or Clinell Clorox bleach wipes work best for both *S. aureus* and *K. pneumoniae*. For larger surfaces requiring liquid cleaning agents, sodium hypochlorite and NaDCC both work well for *S. aureus* with a 60 minute contact time. For *K. pneumoniae*, all the cleaning agents work similarly, allowing for a 60 minute contact time. Here, increasing to a 120 minute contact time did not improve the efficacy of any of the agents.

5.5.2 Mechanical action versus active ingredient

The different wipes were made of different materials, with varying thickness and weave type. It is plausible that the weave type played a role in the removal of organisms. Specific weaves may allow more effective pick up and retention of organisms [371, 372], which is particularly important when preventing the transfer of organisms across different surfaces while cleaning. It has been suggested that optimised cleaning method plays a more important role in reducing surface CFU than the use of a disinfectant rather than a detergent [373].

The Clinell branded wipes both achieved total reductions. The gauze wipe, representing physical removal in the absence of an active ingredient, outperformed alcohol wipes for *S. aureus*, and performed similarly for *K. pneumoniae*. While weave types were not assessed within this study, previous work has considered how gauze may have a superior weave or texture allowing greater organism recovery when compared with other wipes with active ingredients [374]. Testing of different materials and their ability to bind bacteria has revealed that different fiber types bind different organisms with varying efficacy, and that generally, polyester and acrylic fibers outperformed cotton and nylon [371]. Furthermore, size, shape, and inherent charge of an organism itself may play a role. As previously explored in section 3.5.2, size and shape of an organism may allow a surface sampling device to recover some organisms more effectively than others, and that the charge on a sampling device, or in this case a wipe, may help or hinder the cleaning process.

With an effective bacterial-binding weave and appropriate friction while cleaning, the effectiveness of physical removal alone should not be overlooked. With the attractive marketing ploys of antibacterial wipes and how effective they are, it is a concern that the antibacterial properties of the wipes may create a false sense of security, and reduce the effort exerted into the cleaning. Section 6.5.2 discovers how personal perceptions of surface cleaning and how easy a surface may be to clean can have a negative impact on cleaning effort, which may also translate to overreliance in the advertised ‘cleaning power’ of the wipes themselves. As shown within the results, physical removal can achieve a modest reduction of surface bioburden, which is a critical step to break down biofilm and removing organic matter from a surface

to then allow a follow-up disinfection process. This study assessed dried planktonic organisms, not organisms within a dry biofilm, which would provide a greater challenge for the wipes, requiring a strong mechanical removal step in order to break down the biofilm and allow disinfection [300].

Another factor outside of active ingredient enhancing the wipe efficacy, wetness of the wipe was considered [374, 375]. It was found that the wetter wipes performed better than the drier alcohol wipes. However, while the alcohol wipes were found to contain less liquid and be drier, isopropyl alcohol has a greater relative evaporation rate than water, and while the wipes were weighed immediately upon removal from the packaging, immediate evaporation is an issue. Previous studies have also considered the role of liquid content and wipe efficacy, however, this is a complex issue in which 'wetter' does not always mean better, and all wipes have an optimum liquid content depending on the material of the wipe, the target surface to be cleaned, and the organism the wipe was challenged with [374].

There is wide variation in the quality and quantity of information provided by different wipe manufacturers. In light on the lack of training for cleaning in the clinical environment, information provided on how to use wipes should be comprehensive and clear. Staff members may not know where to find their cleaning protocols or have time to look up methods, and without reminder instructions on the packaging of the wipes, they could be used inappropriately. Furthermore, some of the instructions are open to incorrect or ambiguous interpretations, such as instructions for a surface to 'remain treated' for a specific time. The use of the term 'contact time' may not be understood by all types of workers using these wipes without a

microbiological background. Easier to understand terminology should be considered, such as instructions to ensure a surface remains wet with product for a specified time. There are some important components of cleaning not considered by all instructions, such as ensuring visible soiling is removed prior to disinfection and putting appropriate pressure onto the surfaces.

Commercial wipes work differently against different organisms and wetness plays a role. All cleaning agents have variable efficacies across different surfaces. If cleaning agents are used incorrectly, this can lead to poor efficacy or additional contamination of surfaces. Mechanical action is an important step for cleaning and must not be forgotten just because an agent is labelled antimicrobial. Quality of training across healthcare systems is variable and lacking (section 7.5.2) and available manufacture instructions can mitigate risk, though available information can be sparse. This means easy to understand and frequent training and refresher training (section 1.5) as well as 'on-the-go' reminders in forms like reminder cards that slot into NHS badge holders and posters can be valuable (figures 7.2-7.3). These issues and that which were highlighted in the observation and audit study with training intervention [200] (chapter 7) were tackled as part of improvement of cleaning training [200]. Training sessions with the cleaning teams, where real issues and gaps in training and difficulty understanding how to use wipes, were used as a platform for discussing, highlighting, and tackling these issues. These findings show that comprehensive and frequent in-house training is key, as manufacturers usually give a basic suggestive guideline only.

5.6 CONCLUSION

Frequent cleaning of clinical surfaces reduces the risk to patients, by lowering the incidence of HCAI. While cleaning is a vital component of IPC, cleaning protocols vary between different hospitals and are not standardised, therefore different trusts can choose to use different cleaning agents. There are many cleaning agents available for surface cleaning, and knowing which cleaning agent to select can be a difficult undertaking. RTU wipes have grown in popularity for surface cleaning, and are a fast and easy option for cleaning in a busy ward environment. However, cleaning agents, including RTU wipes, are all tested under different conditions, with some testing methods more rigorous than others. Therefore, it is important to know what the manufacturer claims for wipe efficacy translate to for real clinical surfaces.

Wipes and liquid cleaning agents perform differently, and can be comparable under certain conditions. Knowledge of potential surface organisms (determined by ward or patient subset), surface type and contact time can make this selection easier. Testing of the wipes revealed the wipe with no active ingredient performed modestly and outperformed alcohol wipes when challenged with *S. aureus*. This highlights how simple cleaning with detergent and water, using physical pressure to remove organisms, should not be overlooked in favour of more technical or advanced cleaning methods, such as no-touch decontamination systems like HPV, which are growing in popularity for clinical cleaning. These results also demonstrate that different wipes have varying performance profiles and this evidence should inform procurement and use within healthcare settings.

Chapter 6 Cleaning within a Ward – Multiple Factors Affecting Cleaning Efficacy

6.1 Introduction

The role of surfaces in facilitating the transfer of pathogens within the clinical environment is becoming more accepted. Their role as a reservoir for pathogens has been heavily debated, and the surface environment was once considered to play a negligible role. Now, studies have shown that not only can organisms be recovered from the clinical environment [12, 94, 139, 146, 205], survive long-term on surfaces [90, 146], but that a direct link has been found between environmental isolates and clinical infection.

Knowing organisms persist in the clinical environment and how this can lead to HCAIs, it is now clear the surface environment must be considered more closely. It is important to keep these surfaces clean and free of pathogens in order to create a safe clinical environment. Cleaning is the first defence against environmental contamination. Effective cleaning can ensure environmental bioburden and pathogens that could cause HCAI are kept to a minimum. Different hospitals have varying levels and types of contaminants, depending on patient subset, ward type, surface type, geographical location and time of year [219].

Multiple studies have identified that cleaning efficacy is insufficient, either by failure to remove Ultraviolet (UV) markers or remove pathogens, despite these environments often passing by a visibly clean standard, which is the current benchmark for environmental cleanliness in UK hospitals [337]. As such, it is important that hospitals know their environment and have the ability to assess pathogen risk from different surfaces and areas. Assessing cleaning efficacy and

their own environmental microbiome can allow important insight into their unique environment. While routine environmental monitoring is not mandated [337], this can provide an important overview of the normal distribution of contamination within specific ward environments across various ward settings and times. Knowing the normal level and composition of a ward environment will allow detection of anomalous increases or decreases in the regular flora, which could be attributed to increase or decrease in patient turnover, change in numbers of patients infected or colonised with particular organisms, enhanced cleaning, insufficient cleaning, or a potential outbreak scenario. Routine environmental monitoring can also identify potential sentinel screening sites, which can be targeted in the instance of an outbreak, providing an evidence-based initial sampling plan, to reduce the time and resources associated with ward-wide sampling. Results of the following work have been published in the American Journal of Infection Control [317].

6.2 RESEARCH AIMS

The aim of this chapter was to assess the surface contamination of a paediatric ward, and to identify how well cleaning has been undertaken.

1. Assess cleaning within the ward, and identify areas or individual surfaces that were cleaned well, or surfaces that were consistently missed by the cleaning team.
2. Take environmental samples using a method informed from extensive surface sampling literature review undertaken in chapter 2.
3. Use the results to develop an environmental risk assessment for the ward including identification of key control points within the ward, and to better inform cleaning training to improve safety within the ward.
4. Explore factors that affect how well cleaning has been undertaken, such as the surface material, perceived cleanability or perceived risk a surface poses to a patient.

The results from this chapter were published in the American Journal of Infection Control: Surface sampling within a pediatric ward—how multiple factors affect cleaning efficacy [317].

6.3 METHOD

6.3.1 STUDY SETTING

Samples were taken daily over a two-week period from a paediatric haematology-oncology ward at GOSH. The daily sampling plan consisted of 55 sites. A total of 1,160 samples were taken throughout the two-week period. Samples were taken from 12 areas; toilet, sluice, reception seating area, main reception area, corridor, consultation room, outpatient reception area, bed bay 1, playroom, height and weight room, treatment room, cubicle ensuite. The ward consisted of 3 separate 4-bed bays, 3 single rooms with ensuite bathrooms, 4 treatment rooms, a playroom, a height and weight room, 6 consultation rooms, and 2 reception areas with seating for the day unit and outpatients area. The ward layout and composition is shown below in the map.

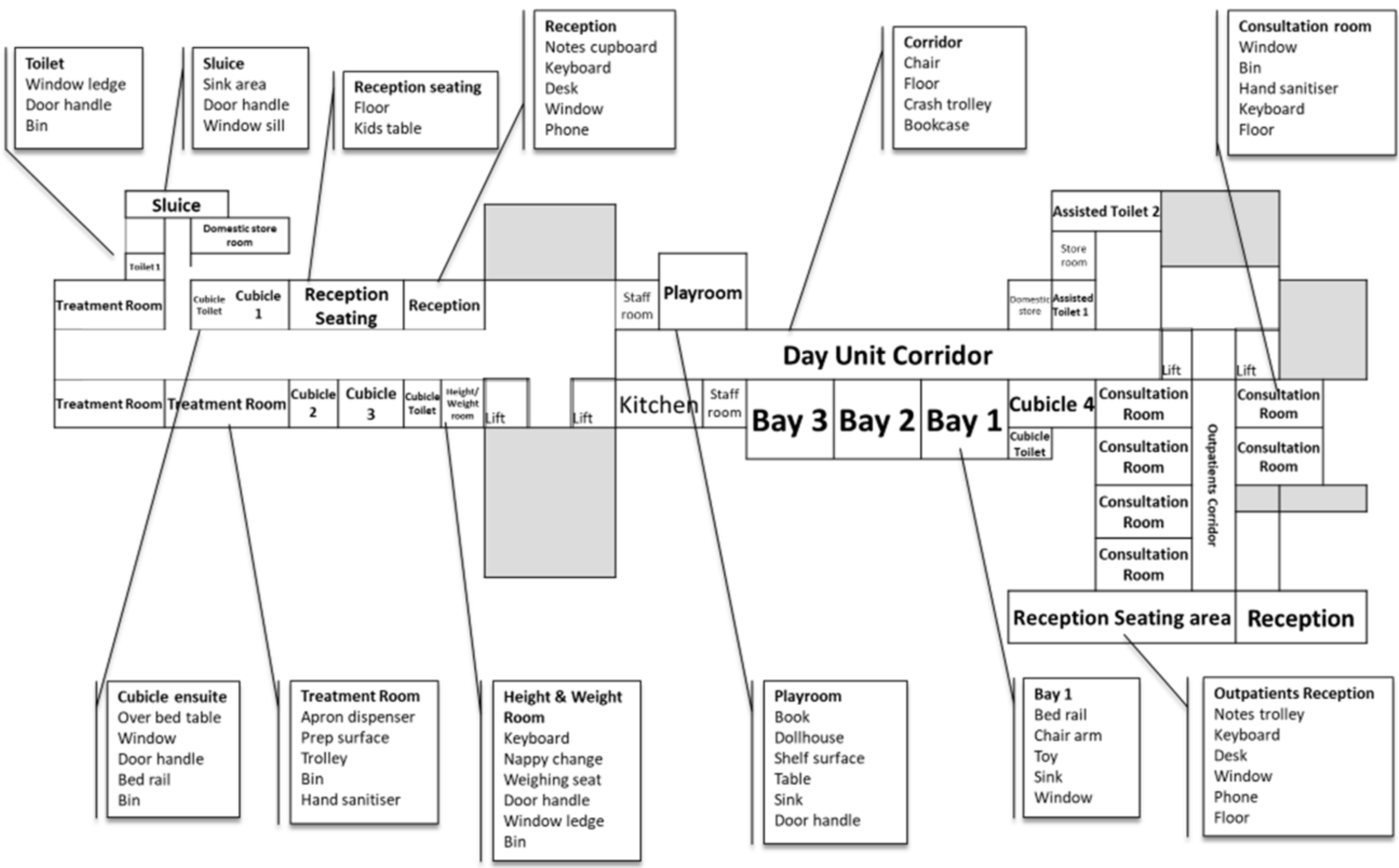


Figure 6.1 Ward sampling map with individual sampling sites

6.3.2 SAMPLING SITES

Sampling sites were selected to form a representation of the ward as a whole, encompassing both clinical and non-clinical surfaces, including near-patient and wider environmental surfaces shared by all visitors within the ward. The 55 sites, selected to represent a range of surfaces throughout the entirety of the ward, across different rooms and between clinical and non-clinical areas including high-touch and near-patient surfaces while also representing some less commonly assessed surfaces such as windowsills and floor areas, were sampled daily before and after cleaning. The sampling plan, as shown in figure 6.1, remained the same throughout the study period. This produced 9 replicates of each sampling site over the 2-week sampling period. To ensure any reduction of organisms seen was due to the cleaning and not removal during the sampling process with the sampling devices, pre-cleaning samples were taken from the left side of surfaces, and post-cleaning samples were taken from the right. This pre-selection of sites allowed the results to determine exact changes on the individual surfaces before and after cleaning.

6.3.3 Surface categories

For the purposes of analysis and assessing trends, surfaces were broken down into different categories. Clinical surfaces are classified as surfaces related to direct patient care.

Table 6.1 Classifications of surface categories.

Surface Category	Details
Surface material	Surfaces are made of a wide range of materials, with different textures, topographies and porosities. Different surface materials can change how easily organisms and different strains of organisms are recovered from a surface, as well as how easily the surface is cleaned. Rougher surfaces will promote biofilm formation, which will require more vigorous cleaning (see Section 3.5.1).
Who interacts with the surface? (N= 55 surfaces)	Interactions were defined as the population subset that comes into contact the most with the surface: all populations, none, patients, or staff. Some surfaces were only touched by clinical staff, such as surfaces within the treatment rooms. Some surfaces were mostly touched by patients, such as the playroom surfaces.
Perceived (by cleaners and healthcare workers) risk to patient	Perceived risk is the assessment of the role a specific surface could play in relation to HCAI: high, moderate or low risk. Surfaces closer to the patient and surfaces known generally to have a greater bioburden represented a greater risk than surfaces within the wider ward environment, or surfaces known to have little contact with the patient. Risk was then allocated following advice from the GOSH IPC team.
Perceived (by cleaners and healthcare workers) cleanability	Surfaces are subconsciously assessed by how easy they will be for a staff member to undertake cleaning: difficult, moderate, or easy. Factors affecting perceived cleanability included size of the surface, surface height, surface material, and general shape of the surface. Surfaces with multiple components, gaps, and crevices, or surfaces in difficult-to-reach areas, such as the reception telephone, were classified as more difficult to clean than smooth, flat surfaces within easy cleaning reach for healthcare workers, such as the reception desk.
Who is responsible for cleaning?	Different staff groups of healthcare workers are responsible for cleaning different types of surfaces. These were broken down into: cleaners, clinical staff or play staff.
Clinical and non-clinical surfaces	Surfaces were divided into the following categories: clinical, bedspace, non-bedspace, non-clinical and sink. Sink surfaces were separated due to the associated increased bioburden.

6.3.4 MICROBIOLOGICAL SAMPLING

Samples were taken a minimum of 3 hours following cleaning to allow any residual chlorine from the cleaning agents to dissipate, so this would not interfere with the recovery of organisms. Cleaning staff cleaned the floors daily with a bleach-based cleaner and mop following closure of the day unit. Healthcare assistants were responsible for cleaning the high-touch surfaces within the height and weight room and used Clinell Universal wipes. During this time, there was no further ward activity or interactions with the surfaces. There was no possibility of recontamination of surfaces or movement of organisms after cleaning as the ward was closed. Samples were taken with 90mm TSA contact plates (Oxoid; Basingstoke, UK). Plates were pressed against the surface for 10 seconds with firm pressure, and incubated at 37°C for 24 hours. Following incubation, CFUs were counted.

6.4 RESULTS

Data are presented as CFU per plate. All statistical analysis was undertaken in GraphPad 7 software (San Diego, CA).

6.4.1 CLEANING EFFICACY

A total of 1,160 contact plate samples were recovered from 55 sites daily before and after cleaning. Before cleaning, recoveries ranged, on average (mean), from 0.44-115.33 CFU/plate. Following cleaning, recoveries ranged from 0-80 CFU/plate. On average, cleaning allowed a 68% reduction in surface CFUs, which was a significant reduction ($P < .0001$). Figure 6.2 below shows the comparison between CFU before cleaning and percentage change after cleaning. A clear relationship can be seen, where surfaces that were highly contaminated before cleaning (judged as >50CFU/plate) had an increased reduction in CFU after cleaning, when compared to their cleaner counterparts. For surfaces that were less contaminated, there was a more frequent risk of recontamination either due to cross-contamination during cleaning or contamination once the ward re-opened and cleaning was missed, with some surfaces starting at <20CFU/plate becoming contaminated by an increase of up to 180%. This was seen in surfaces such as the day reception desk, at 25.50 CFU/plate before cleaning, increasing to 61.60 CFU/plate, the outpatient reception phone starting at 10.90 CFU/plate increasing to 21 CFU/plate, and the bin in toilet 1 increasing from 1.10 CFU/plate to 18.80 CFU/plate following cleaning.

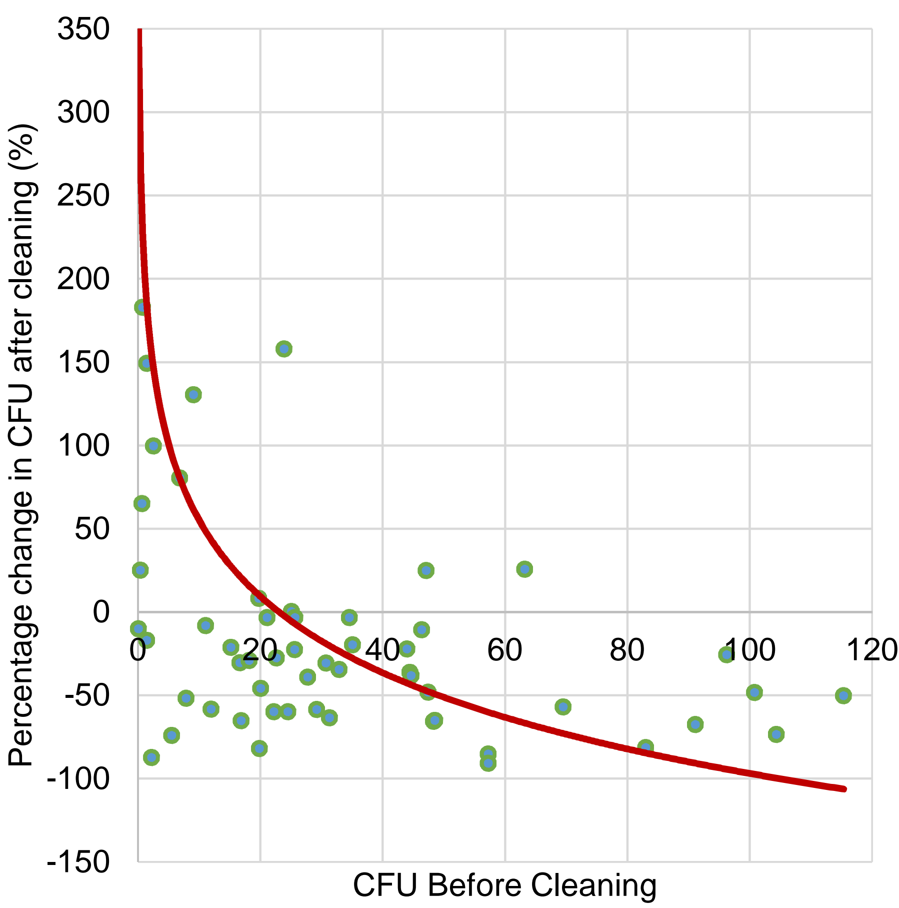


Figure 6.2 Relationship between mean before cleaning and mean percentage change of sampling site after cleaning (N= 55 sites) (Rawlinson et al. 2019).

These results support the theory that often, when cleaning, contamination is being spread throughout the ward, either due to ineffective technique (demonstrated in an observation study in a separate ward chapter 7), or use of contaminated cleaning equipment (which has demonstrated, in the literature, as a problem see section 5.5.1) or insufficient concentration of cleaning agent (section 5.5.1). Observations were not undertaken during preparation of cleaning agent, cleaning technique, and cleaning equipment were not tested so it cannot be determined where the spread occurred, though the relationship between the

percentage change of CFU before and after cleaning show at some stage, this spread is occurring.

6.4.2 Cleaning perceptions and the impact on CFU

The results were further divided into categories to assess potential human and non-human factors that could cause variability in cleaning. Personal perceptions such as 'cleanability' risk to patient, surface material, who cleans and who interacts with a surface all had an impact on cleaning efficacy.

6.4.2.1 Who cleans?

Difference surfaces are allocated for cleaning to different staff members. Surfaces cleaned by cleaners, clinical staff or play staff were divided and explored. The results found that, on average (mean), surfaces cleaned by play staff had increased loading (27.93%), when compared with cleaners, (19.22%) or clinical staff (19.15%). No statistical significance was found between groups; play staff and clinical staff (P=1.000) play staff and cleaners (P=.840) or clinical staff and cleaners (P= .873).

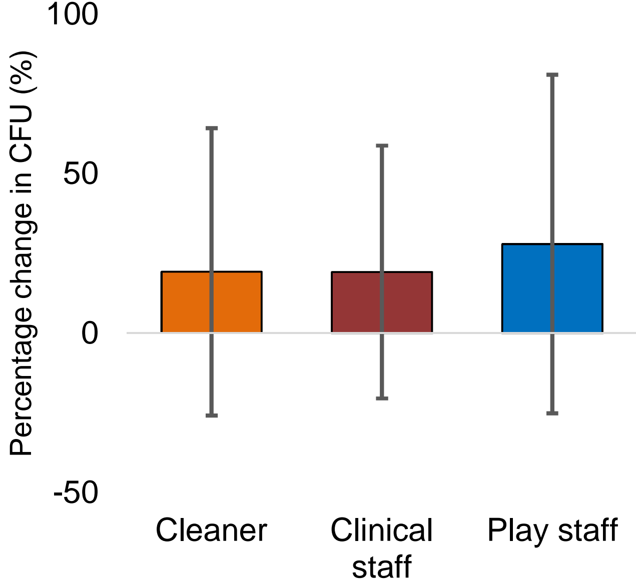


Figure 6.3 Mean percentage change in microbiological contamination of all surfaces sampled (N= 495 samples) within the study divided into staff group undertaking the cleaning.

6.4.2.2 PERCEIVED CLEANABILITY

The cleanability of a surface, or the personal perception of how easy a surface was to clean, was shown to play a role in how well a surface was cleaned. Perceived cleanability and how ward staff perceived their surfaces was determined upon informal discussions with the cleaning teams and healthcare workers on the ward. The results found that surfaces classified as easy to clean had the greatest increase in CFUs following cleaning, (34.13%) when compared with surfaces classified as difficult to clean (18.31%). Surfaces with moderate cleanability had an average (mean) reduction of 16.66% in CFU. This shows personal perceptions of how easy surfaces are deemed to clean has an impact on the number of CFU remaining on the surface after cleaning. No statistical significant was reported across the groups, between difficult and moderate to clean surfaces (P=.946), difficult and easy to clean surfaces (P=.988) or easy and moderate to clean surfaces (P=.934).

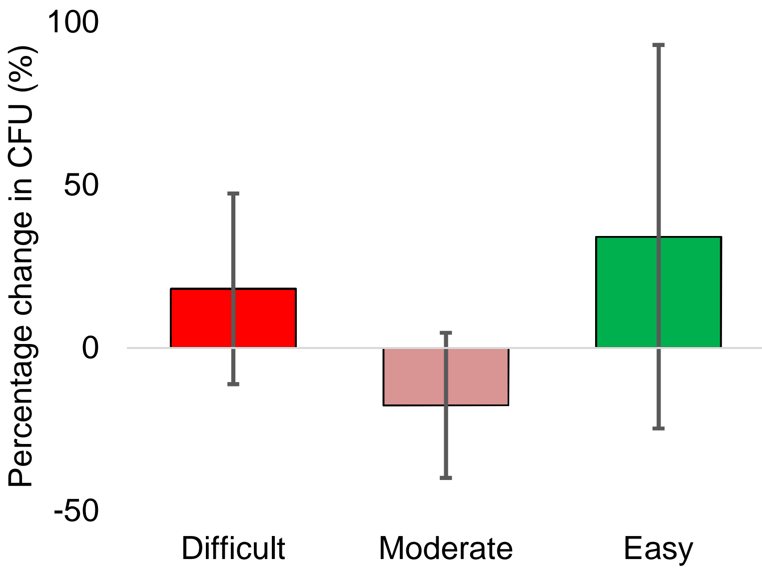


Figure 6.4 Mean percentage change in microbiological contamination of all surfaces sampled (N= 495 samples) within the study divided into easy, moderate and difficult to clean surfaces.

6.4.2.3 SURFACE MATERIAL

Surface material was shown to have an impact on the CFU recovery after cleaning. Metal surfaces (N= 14) had the greatest impact, with an increase in CFUs of 167.68%. Ceramic surfaces (N=2) had the most reduction in CFU following cleaning, with a reduction of 77.18%. Vinyl (N =3), wood (N= 10) and plastic surfaces (N= 26) also had modest reductions in CFUs, with 28.04, 17.17 and 4.05% reductions in CFU respectively. No statistical significance was reported between groups.

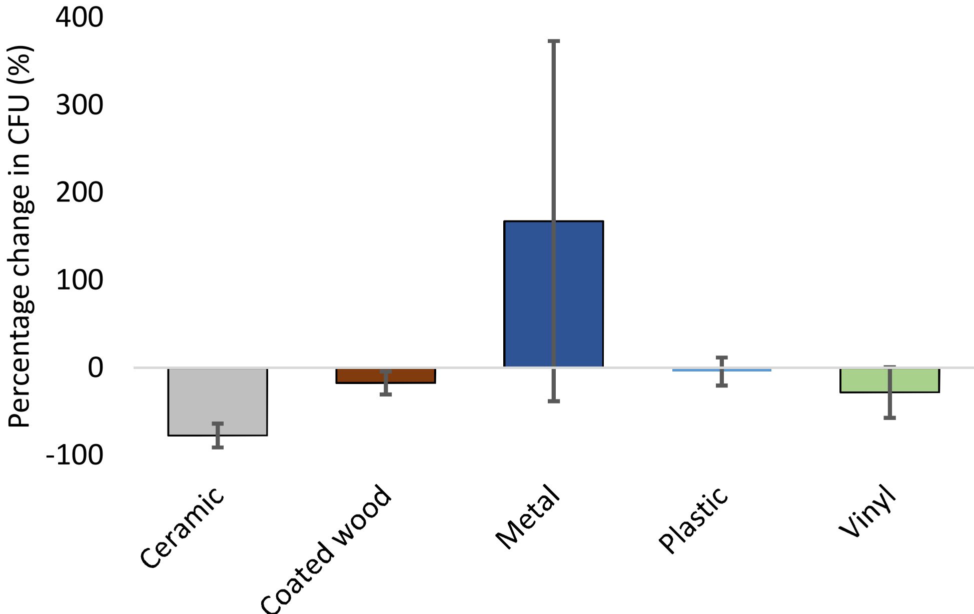


Figure 6.5 Mean percentage change in microbiological contamination of all surfaces sampled (N= 495 samples) within the study divided by surface material.

6.4.2.4 INTERACTIONS

The results showed that the populations interacting with a surface impacted the recovery of CFUs following cleaning. Surfaces that were interacted with by all populations moving within the ward environment had a 119.88% increase in CFU following cleaning. This is in contrast with surfaces with mostly patient interactions (-21.59%), staff interactions (-4.45%) or no interaction (-29.44%), which all had reductions. No statistical significance was reported between groups.

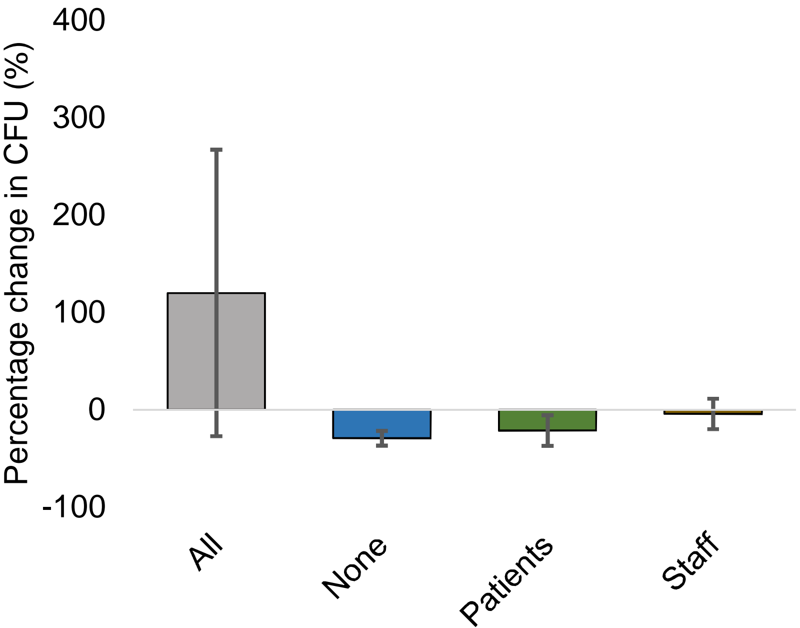


Figure 6.6 Mean percentage change in microbiological contamination of all surfaces sampled (N= 495 samples) within the study determined by the population subset interacting most with that surface.

6.4.2.5 RISK

Surfaces were classified into high, moderate and low risk to a patient. High risk surfaces were the only category of surfaces to have a reduction in CFU following cleaning, with 17.87% less CFU post clean. The moderate and low risk surfaces were not cleaned as effectively, and both had increases in CFUs following cleaning, at 18.21% and 35.28% respectively. No statistical significance was reported between groups of high and moderate risk (P=.373) or high and low risk (P=.311) or moderate and low risk surfaces (P=.424).

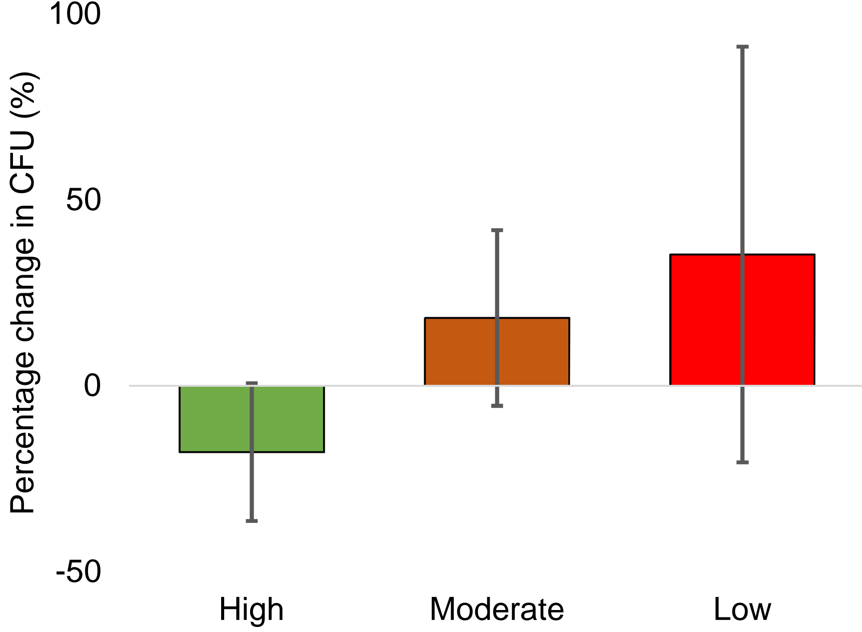


Figure 6.7 Mean percentage change in microbiological contamination of all surfaces sampled (N= 495 samples) within the study by perceived risk to a patient, divided into high, moderate and low risk.

6.4.3 WHAT DOES THIS MEAN AGAINST CURRENT PROPOSED STANDARDS?

If the surface samples were assessed against the two currently available proposed standards for clinical surface sampling, all of the surfaces would be classified as microbiologically safe under the Dancer standard, both before and after cleaning, as shown in figure 6.8 below. Under the Griffith standard, however, ten of the surfaces would fail before cleaning, and three would fail after cleaning.

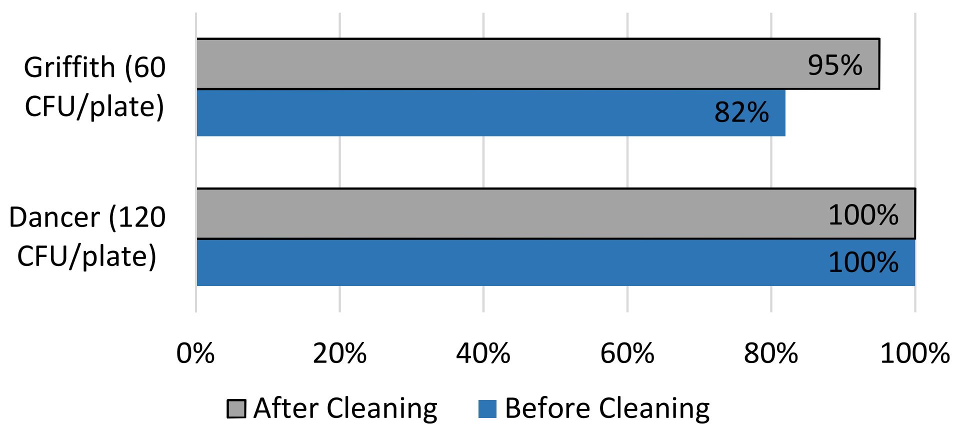


Figure 6.8 Percentage of surface samples determined to pass when assessed against the Dancer and Griffith standards for clinical surface sampling.

Under the Griffith standard, before cleaning, the playroom shelf, toilet door handle, cubicle window sill, the sink in bay 1, corridor chair arm, three floor samples (corridor, main reception seating area, outpatient reception seating area), the corridor bookcase and a main reception phone sample would fail. Following cleaning, the outpatient seating area floor and corridor chair arm surfaces would still fail, with the addition of the main reception desk.

6.5 DISCUSSION

This study sought to assess how well cleaning had been undertaken within a paediatric ward. Due to the study setting and closure of the ward prior to evening cleaning, it was possible to assess the direct impact of cleaning on surface bioburden, by taking samples before and after the daily cleaning had taken place. The results found that across the 11 areas sampled within the ward, there was a wide variation in the level of CFU recovered from the different surfaces, and how there are multiple factors that can impact cleaning effectiveness, such as the surface material, the staff group delegated to clean, the population subset interacting with the surface, and perceived cleanability. As cleaning is a critical component to keeping surfaces clean and reducing HCAI risk, exploring how different surfaces are associated with different levels of bioburden is important for developing effective cleaning training programmes, as well as highlighting the role of personal perceptions and other factors that can have an impact on how well cleaning is completed. With the rise of multi-drug resistant organisms (MDROs) and HCAI, soon, cleaning may be the only defense against MDROs.

Many studies have shown how cleaning efficiency is variable, and have shown varying success with cleaning training interventions to reduce surface contamination in hospitals, with an overall goal of reducing HCAI [191, 376, 377]. The results found that overall, cleaning was moderately effective throughout the ward. On average (mean), cleaning produced a reduction of CFUs by 68%. Surfaces with lower CFUs before cleaning were found to become more contaminated after cleaning, by up to 180%, as shown in figure 6.2. Several factors were explored and were found to have an impact on cleaning procedure and bacterial loading of the surfaces.

6.5.1 WHO CLEANS THE SURFACE?

Different staff members are delegated to clean different types of surfaces. Play staff are responsible for cleaning items within the playroom. Cleaners are responsible for cleaning the wider ward environment and permanent fixtures, such as walls and floors, while nurses are responsible for cleaning the surfaces within their clinical space, such as the IV trays, equipment trolleys, and objects on the nurses station. The results showed that the staff group who is delegated to clean had an impact on how well the cleaning had been undertaken. When assessing average (mean) CFU recovery over the entire study period, surfaces allocated to clinical staff and play staff for cleaning had the lowest CFUs at 8.13 CFU/plate and 12.77 CFU/plate respectively after cleaning. Surfaces allocated to cleaners had higher CFUs at 37.38 CFU/plate. Cleaners cleaned the ward environment once per day and were allocated several hours dedicated only to this role, and should therefore have the most CFU reduction as cleaning forms the entirety of their role. However, cleaners are not just dedicate to one area unlike other staff members and must clean the entire ward. Play staff are only in charge of the playroom, and have a more limited number of surfaces that require cleaning. Clinical staff, as assessed in chapter 6, had a deeper understanding of the science behind cleaning in comparison with cleaners who had not been taught this, and had the need to spot-clean their areas more frequently, though not always the time within their busy role to comply with this. Additionally, clinical staff and cleaners have different understandings of IPC and pathogen transmission, and will have varying understanding of the science behind cleaning. Understanding why cleaning is undertaken is important for high quality, effective cleaning. Many intervention studies to improve cleaning are based on, or

have a main component of training to ensure understanding [159, 191, 198, 377, 378]. These studies have shown how even simple training using a UV marker can help cleaning teams understand the importance of proper cleaning to remove a surrogate for infection, allowing them to better visualise their environment.

6.5.2 PERCEIVED CLEANABILITY AND RISK TO PATIENT

The 'cleanability' of a surface was classified as the personal perception of how easy a surface was to clean. This perception was found to have an impact on how well the cleaning was undertaken. Easy to reach surfaces, surfaces without different components and smooth and flat surfaces were classified as easy to clean. Surfaces that were classified as easy to clean, such as a desk or a door handle, had the greatest increases following cleaning with 34.13% increase in CFU. The surface classified as difficult to clean, such as out of reach tall shelves, or surfaces with multiple components like a dolls house still had an increase in CFU, at 18.31% after cleaning, though less so than the easy to clean surfaces. This is interesting, as smooth surfaces are known to be more difficult for bacterial attachment and are easier to clean (section 3.1). This suggests that the perception of the staff member is impacting how well or how much effort they expend in cleaning and they are focusing more on difficult to clean surfaces as they have a preconceived notion that they will have to work harder in order to fulfil proper cleaning.

High-risk surfaces were found to have lower CFUs (14.60 CFU/plate) than moderate risk (24.04 CFU/plate) and low risk (41.07 CFU/plate) surfaces, with high risk surfaces being cleaned most effectively, with a 17.87% reduction in contamination. In contrast, moderate and low risk surfaces had an increase in

contamination following cleaning, at 18.21% and 35.28% increases respectively. This suggests due to the high risk surfaces being considered more closely, they are being cleaned more effectively and frequently than the surfaces classified as moderate and low risk. This implies greater cleaning effort is being exerted onto the surfaces thought to be difficult to clean, while less cleaning effort is used for the surfaces deemed easy to clean.

The risk to the patient was classified as the personal perception of what an individual surface posed to a patient. High-touch surfaces and near-patient surfaces are classically considered the most high risk. Their constant use and proximity to the patient are a factor that increases the risk of pathogen transmission. As such, these high-touch surfaces are often the focus of enhanced and more frequent cleaning, as well as a target for surface sampling to ensure patient safety [379-382]. This study found that contamination was distributed across the ward, and that the highest levels of contamination were found in the corridor and the reception areas, while other surface sampling studies have reported the near-patient surfaces to be the most contaminated, such as bed tables and bed rails [126, 258, 383].

The increased contamination in the wider ward areas could be due to a variety of factors, such as increased traffic in these areas, variation of interactions with these surfaces, and different staff groups allocated to clean these surfaces, as well as the potentially increased concern for near-patient surfaces leading to enhanced and more frequent cleaning, to the detriment of surfaces perceived as lower risk, such as areas in the wider ward environment, such as reception spaces and waiting areas.

6.5.3 SURFACE MATERIAL

The results found that surface material had the greatest impact on bacterial loading when compared with the other factors addressed within this study. Metal surfaces including apron dispensers, waste bins, fan, trolleys, bed rails, and sluice sink had a 167.68% increase in contamination following cleaning, despite on average (mean), having the lowest CFU when compared to the other surface materials (14.66 CFU/plate). This could be due to metal surfaces being perceived as easy to clean, and as previously discussed, this perception can lead to poorer cleaning and therefore greater CFU. Figure 6.2 also shows how surfaces with lower bioburden prior to cleaning were often found to be more contaminated following cleaning. Before cleaning, vinyl (floors) surfaces had the highest average CFU, at 70.66 CFU/plate. Coated wood (windowsills, shelves, bookcase and chair arms) and ceramic surfaces (sinks) also had higher CFUs, at 49.78 and 44.29 CFU/plate. Plastic surfaces (toys, keyboards, fireproof door handles, phone, desk surface, had a relatively low CFU, at 24.89 CFU/plate. While ceramic surfaces were found to have higher CFUs when compared with coated wood and vinyl, ceramic surfaces had the most reduction during cleaning, with a reduction of 77.18%. In comparison, vinyl, coated wood and plastic surfaces only achieved an average 28.04, 17.17 and 4.05% reductions respectively. This implies that some surfaces are both easier to clean, as well as easier to contaminate. Furthermore, organisms are more readily recovered with sampling devices from some surface materials when compared with others, as found in section 3.5.1, in which organisms were most easily recovered from ceramic and metal surfaces when compared with plastic. The ability for organisms to form biofilm on different surfaces is also addressed, which will play a role in how easily

they are cleaned from the different surface materials. As explored in chapter 3, organisms adhere more easily to different surface materials, and strong biofilm formation, promoted by certain surfaces, will require vigorous cleaning with a detergent to break down the biofilm and allow proper cleaning to take place.

6.5.4 INTERACTIONS

The distribution and level of contamination in relation to interactions with a surface was also investigated. Surfaces were broken down into contact by all ward users, none, patients, or staff. These categories were determined by informal observation of the ward environment. It was found that surfaces with the most interactions with patients, including parents and guardians, were the most contaminated following cleaning. Surfaces within the height and weight room had consistently lower levels of CFUs when compared to other areas, despite this area being in constant use throughout the day, and the nature of the surfaces having a potential for heavy contamination, such as the nappy changing table. Due to the perceptions of these surfaces potentially being high risk, as they had increased contacts with patients and were within a clinical space, this could have contributed to the lower CFU's, as the results showed how higher risk surfaces were cleaned more effectively than surfaces perceived to be low risk. When assessing average overall contamination, surfaces with patient interactions were the most contaminated, with 50.13 CFU/plate. For percentage change following cleaning, as would perhaps be expected, surfaces that were interacted with by all ward users had the greatest increase in CFU (119.88%) which is likely correlation, not causation.

The paediatric setting makes the patient interaction with surfaces unique. Paediatric patients behave differently to adult patients, and will therefore play and interact with surfaces as they usually would in their daily life. This will attribute to the increased loading on the surfaces they interact with. Paediatric patients represent a different source of contamination when compared with adult patient, linked to have skin contamination with faecal flora linked to nappy wearing, or increased viral loading on the skin from infections endemic to the paediatric population, such as adenovirus infection [254].

Additionally, surfaces that would potentially be less of a concern in an adult setting due to the lack of patient contact, such as floors and walls, become a new challenge in a paediatric subset, as children crawl, touch, and play interact differently with these surfaces [384]. Floors are usually dismissed from microbiological sampling [385, 386], and some even advocate for reduced cleaning of floors to focus on other areas, rationalised by the limited interaction between adult patients and the floor surfaces [385]. Though even in an adult setting, there is still interactions with the floor, as things are dropped on the floors and picked up, handbags are placed on floors [387], and sometimes patient falls occur, particularly in geriatric settings.

The interactions between the clinical surface environment and the ward users is dynamic, and an observational study by Cohen *et al.* revealed that of all traffic entering a patient room, 33% had a touch interaction with only the environment [388].

Surfaces with most frequent staff interactions had the lowest contamination, with 24.42 CFU/plate. It is unsurprising that staff member contacts could potentially lead to less contamination when compared with other ward users, as staff have an increased understanding in training and IPC. Additionally, clinical staff will have increased interactions with clinical surfaces compared with other ward users. These clinical surfaces will be associated with more frequent spot cleaning with disinfectant wipes before and after use, such as for prep surfaces, trolleys, or nappy change areas as cleaning these surfaces is a fundamental component of the role of a healthcare professional prior to use, as well as hand hygiene. Compliance with this component could be linked to perceived risk as the clinical staff member is usually cleaning these surfaces prior to patient contact, and know these surfaces are frequently used in relation to patient contact, and will therefore be of the understanding that there is direct risk to patient as they or colleagues provide care. This is in contrast to areas that are usually only spot cleaned when required (showing visible soiling) such as walls, floors or window areas. While these surfaces cannot be forgotten, and form an important component of the hospital surface environment, they are inherently lower risk to patients than other surfaces that come into direct contact with a patient, such as the nappy change table, or surfaces that clinical staff may touch before or during patient care, such as door handles, keyboard in the height and weight room, or treatment room surfaces. The end goal of all cleaning is preventing true risk to the patient by allowing safe care.

Clinical staff, such as nurses, are continually performing hand hygiene throughout the day, and donning and doffing gloves between patient contact and

environmental surface contact, which would lead to reduced contamination of surfaces. Interestingly, surfaces with no contact with any ward users had 36.46 CFU/plate, potentially due to their low risk consideration. These surfaces had a reduction in CFU following cleaning, with a reduction of 29.43%, which can be attributed to the lack of direct re-contamination following cleaning, due to no contact.

The results suggest all ward users are responsible for transferring organisms across the surfaces. All traffic through the ward can contribute to the transfer and deposition of organisms, but clinical staff are of special concern as they come into contact most often with the patients, and perform high-risk procedures that break the skin and provide opportunity for infection. This movement of infection and facilitation by people is explored more in chapter 7.

6.5.5 PERSONAL PERCEPTIONS AND THEIR IMPLICATIONS FOR CLEANING AND CLEANING STAFF TRAINING

Several of the factors assessed within this study were based on personal opinions and perceptions of cleaning and how their role is important to the clinical environment as a whole. The risk a surface poses to a patient and how easy a surface is to clean has revealed to have an impact on how well the surface is cleaned. Higher risk surfaces are cleaned more effectively, potentially to the detriment of other moderate and low risk surfaces. Surfaces classified as difficult to clean had the lowest CFUs, and surfaces classed as easy to clean had nearly double the number of CFU/plate than their difficult to clean counterparts. This could be the result of an individuals perspective of cleaning, and how cleaning should be done in order to

be most effective. As cleaning training is lacking and inconsistent, and often performed 'on the job' by a colleague, these personal perceptions having an impact on cleaning can be continued through many generations of cleaning staff. Staff groups with increased understanding of IPC, such as clinical staff, their personal perceptions of surfaces can be a more evidence-based approach, and more likely to result in better cleaning, though this is not necessarily always the case. Lack of understanding has been found even in clinical staff groups as explored in chapter 7. Therefore training to guide personal perceptions in an evidence-based IPC manner rather than common sense and using techniques one might use to clean their home, is critical to ensure cleaning is complete in a way that is appropriate for the clinical space to prevent the spread of contamination and reduce the incidence of HCAI.

As personal perceptions, the 'human components' have such a large role in how well cleaning is undertaken, this must be considered when designing and producing cleaning training and training interventions for the clinical environment [333]. Feeling undervalued and a lack of understanding of IPC principles due to poor teaching or training leads to poor cleaning compliance [378]. Cleaning is only as good as individual compliance, so considering the weight of personal perceptions of surfaces, this could prove an effective target for training. Including an overview of IPC, teaching why cleaning is undertaken in a specific way in order to reduce the spread of organisms and clean effectively, as well as the particularly important role that cleaners play in preventing HCAI can produce good results in training interventions (Chapter 7).

6.5.6 WHAT DOES THIS MEAN UNDER CURRENT PROPOSED SURFACE SAMPLING GUIDELINES?

This study considered surface CFUs and reduction of CFUs after cleaning in order to consider how well a surface has been cleaned. Microbiological surface sampling is not currently mandated for hospital surfaces outside of outbreak, and the current guidelines are to clean to a standard that produces visibility clean surfaces, under the National Specifications for Cleanliness in the NHS (NHS 2007). The results were assessed against the available proposed standards for the clinical environment, allowing 120 CFU/plate under the Dancer criteria [212] and a more conservative 60 CFU/plate under the Griffith criteria [213], as shown in figure 6.8. However, these standards are for bacterial CFU, and do not have a viral counterpart. Like ATP and UV testing, these guidelines are not specifically related to CFU number, but can still provide some insight into the efficacy of cleaning as a whole, which will have an impact on reducing loading of all microbiological contaminants, including viruses [389]. Though the numbers are only in relation to general reduction of contamination on a surface and cannot be used for risk assessment as we must consider infectious dose, how well viruses can survive in the environment and size, as *S. aureus* is approximately 1µm in size and in contract, norovirus is 38nm so CFU limits of bacteria cannot transfer in terms of risk. This is particularly relevant when assessing a paediatric setting, due to the large proportion of viral infections in hospitalised children [384] and the associated increased viral loading. The differences between these two proposed assessment criteria highlight how determining the safety of the clinical surface environment can be difficult and confusing, particularly when the current standard is for visibly clean only. Using the stricter Griffith criteria, after cleaning, 52 of 55 could be classified as safe, and of the 3 failures, only 1 can be

classified as a high touch surface. Several of the failed samples were floor surfaces, and generally floors are excluded from clinical surface sampling as they are not deemed important [385, 386].

However, more work needs to be done to produce a more robust set of guidelines for clinical surface sampling [139] to allow staff to identify and determine the safety of their surfaces and if cleaning had been undertaken well in relation to risk assessment. The Griffith and Dancer criteria is not achievable across all hospital surfaces or surfaces in different areas of the hospital. An extensive 2- year sampling study of a NHS hospital found that >60% of near patient surfaces had an average of 380000 CFU/cm^2 [390]. In an ideal scenario, all surfaces within the hospital would pose no risk to patients. To determine surfaces as posing no risk, they would need to be sterile. This is not practical or possible due to the nature of the environment, therefore the standards used need to be assessing the individual risk of different surfaces, not just general CFU or total viable count (TVC) limits. On a practical side, it must be understood that whole-ward cleaning cannot be a constant and never-ending process [391]. There are limits to the amount of cleaning a single cleaning team can deliver within a day, limits to the individual budget a hospital reserves for cleaning and IPC, and overburdening a cleaning team could result in poorer compliance due to overwork in order to reach certain criteria. Enhanced cleaning can only be a positive step and these measures have been successfully linked to reduced environmental contamination and reduction of HCAI [132, 392]. A study by Dancer *et al.* 2009 found introduction of an additional cleaner led to 32.5% reduction of microbial contamination and a 26.6% reduction of *S. aureus* acquisition by patients,

saving the hospital between £30,000-70,000 [132]. However, these enhanced interventions do not always yield significant impacts for all settings on patient acquisition of clinical infection, such as MRSA, therefore the standard set needs to reflect the implementation of only the most effective cleaning to give the best outcomes which is the fundamentals of cleaning; a cleaner, safer environment leading to reduction of HCAI [393].

The Griffith and Dancer criteria make an important contribution to providing microbiological pass and fail standards for surface sampling, yet these guidelines are not evidence-based. A high surface bioburden does not necessarily mean a surface is unsafe or would lead to clinical infection. A more precise evaluation of surfaces would be to search for a range of clinically significant pathogens. However, CFUs can give a good indication of cleaning. This quantification of number of organisms present on a surface is valuable as it allows trend analysis of specific surfaces and rooms, and can be linked to specific incidences, such as introduction of new cleaning policy, introduction of training or increase in patient turnover.

The nature of some surfaces will lead them to have a higher bioburden, such as floors. However, due to the paediatric setting and the different interactions the patients and siblings have with their environment, the standard near-patient environment should not be the only consideration. Paediatric patients often have an increased interaction with lower surfaces such as floors and walls, which would not be the case in a general adult setting.

6.6 CONCLUSION

This study sought to assess how well cleaning had been undertaken using microbiological surface sampling before and after cleaning had taken place within a paediatric day ward. The results found that, overall, cleaning produced a reduction of surface contamination of 68%. Some surfaces were consistently cleaner than others, both before and after cleaning, such as surfaces in the height and weight room. These results highlight that not all surfaces can be held to the same standard, and as different surfaces pose different risk to the patient, these results should be considered when developing environmental monitoring programmers. Monitoring of individual clinical environments to build a picture of the general microbiome is discussed as an important step (section 6.1). Not all benchmarks and proposed standards will fit all hospital areas, therefore assessing a specific environment before determining and applying a benchmark is key. The results from this ward sampling study became a legacy impact for this thesis, and continue to be used at GOSH to keep discussions ongoing to improve how cleaning is assessed as in-house benchmarks are needed to keep control of external cleaning contractors in light of poor performance. These results were also used to inform future training implemented on that ward and when designing the training protocol for the cleaning training intervention in the following chapter, to highlight how each surface must be considered in its own right as different surfaces have different properties, risk, and contamination levels as an evidence-based way to present ideas to the team.

Some surfaces were found to be more contaminated, such as floors and the bookcase in the corridor. The individual impacts of different factors were assessed,

and it was found there are a multitude of factors affecting cleaning efficacy. Where the surface is located, the material the surface is made from, the population subset interacting most with the surface, the staff group responsible for cleaning, the perceived risk to the patient and perceived cleanability all played varying roles in impacting the bioburden and how well the cleaning had been undertaken. This study highlights how there are many factors impacting cleaning, and these must all be considered when designing cleaning training programmes in order to be effective and produce long term improvements.

As previously discussed, there is a distinct lack of standards and guidelines for environmental monitoring of clinical surfaces [139]. This study used the two current proposed standards. While these standards are not evidence-based, they provide an important benchmark for assessing samples, with a distinct pass or fail criteria in the form of CFU limits, allowing easy interpretation of a sample. Under the Dancer criteria [212] all samples within this study would pass, as be classified as clean. Under the Griffith criteria [213] just 3 of the surfaces would fail. When considering the vulnerable paediatric subset, the implications of this require further discussion, and there needs to be more work and discussion on setting microbiological limitations based on evidence.

Overall, cleaning was acceptable, though some surfaces were consistently missed, as demonstrated by little to no change in CFU over the sampling period. As found in section 7.5.2 and the wider literature, cleaning training has been found lacking. The findings from this study can be used to develop training programmes for more effective cleaning. Knowing how personal perceptions play such an important

role on how well cleaning is undertaken, these components can be used as a target for training interventions, to improve compliance and cleaning practise, leading to more effective cleaning and safer surfaces in the clinical environment. This discrepancy between cleaning quality and staff group will be explored in the following chapter in which cleaning compliance of different staff groups (cleaners and nurses) is assessed.

Chapter 7 OBSERVATIONS OF CLEANING AND TRAINING INTERVENTION

7.1 INTRODUCTION

Human behaviour and personal perceptions of responsibility within a professional role are difficult factors to change and control, despite this 'human component' having a significant impact on cleaning and infection transmission [317, 394] [333]. The role of people has been proven in the transmission of pathogens from surface to surface [395]. Anyone moving within the hospital and interacting with the space will facilitate the movement of organisms, including visitors, patients, domestic staff and healthcare workers. This movement and transmission is inevitable, yet the contribution of behaviour is not well explored. For example, hand hygiene is an important component of infection control, yet relies entirely on an individual's compliance. Achieving good compliance for hand hygiene is a commonly recognised issue in healthcare settings across the world, [396-398] and many schemes have been put in place specifically to improve compliance, though often to mixed success. One factor proven successful, however, is the availability of a good role model, particularly when that role model is a senior member of staff [394, 399]. Therefore, it would be reasonable to suggest the same dynamic could apply to surface cleaning.

As previously discussed in chapter 4, cleaning of the surface environment is vital to limit HCAI. Despite the importance of effective cleaning, this important infection prevention tool is often overlooked. During a busy clinical day, for clinical staff groups, cleaning can be overlooked in favour of immediate clinical tasks, or other jobs that would immediately affect patient safety. While the threat of

antibiotic-resistant organisms is well known among all healthcare workers, this environmental contamination cannot be seen, and the threat it poses to patient safety is not visible until infection or outbreak has occurred. With the ever-stretched burden on the NHS and lack of time and resources, cleaning training and cleaning competency is often left lacking.

Poor cleaning compliance can be caused by a multitude of factors, including personal perception, quality of training received, frequency of re-training, how valued domestic workers feel, availability of role models and allocated time and resources for cleaning training [394, 399]. A study by [378] demonstrated poor compliance can be attributed to domestic workers feeling undervalued, as well as a lack of understanding of basic infection control principles.

It could, then, be determined that clear, regular training is the key to improving cleaning compliance and quality. However, there are multiple issues with implementing effective cleaning training. There is little guidance on how to train and how often to re-train, and what constitutes a well-rounded and effective cleaning training package for both domestic or clinical cleaning [201, 400]. Different hospitals choose to train their staff differently and, with the rise of subcontracted cleaners, training is often not undertaken in-house, and therefore the quality of training given to the staff is often unknown.

Many studies have addressed this issue and used large, multifaceted training interventions in order to improve cleaning within the clinical environment. These studies vary in the types and scale of interventions used, as well as the methods used to assess their impact [159, 197, 198]. While large-scale training interventions are

effective, they are often not practical or feasible to roll out in some clinical environments. They are resource heavy, and require investment of both time and money which not all hospitals have at their disposal. The training designed for this study sought to produce a small, yet carefully targeted training intervention to maximise efficacy while remaining a feasible training package that could be used in the clinical environment.

The practicality of training is important, as different staff members have different roles in relation to cleaning. Nurses are required to undertake cleaning of their clinical surfaces before and after use as needed, as well as their larger nursing role. Cleaners are dedicated to cleaning only, including general cleaning of the wider ward, though their day-to-day schedule can vary significantly. Some days may require multiple discharge cleans or infectious cleans, depending on current patient occupancy.

Bundle-style training with audit and re-audit are commonly used within the infection prevention and control (IPC) setting, and are an important tool for improving practice [401, 402]. A common IPC bundle format training intervention is the ‘5 moments for hand hygiene’ campaign [403]. Bundle training is based on 3-5 elements [404] implemented together as a set, which can attain greater improvement that if they were used alone. For this study, the 5 bundle components were chosen by assessment of the literature and determining what constitutes as good practice to improve cleaning, such as using the correct number of wipes while cleaning, and ensure the correct wipe has been chosen [405-408]. The bundle components were designed to be applicable across different staff cleaning in the

hospital as findings from 6.5.1 informed how different staff subjects may perform differently, and would also need to be assessed separately, and that the bundle would need to be designed in a format where different components can be delivered individually in relation to compliance across nurses or cleaners.

An observational study was undertaken to produce an audit tool to assess cleaning compliance against a pre-defined bundle based on the best current evidence available within the literature and consultation with IPC professionals. The 5-part cleaning bundle was defined in order to audit and give a score to each cleaning opportunity. Based on the results of 50 hours of cleaning observations, a targeted cleaning training package was designed and given to all staff within the ward. Following the training, a further 50 hours observations were undertaken, and cleaning was audited against the same 5-part bundle to assess scores before and after targeted training.

7.2 RESEARCH AIMS

The aims of the work presented in this chapter were to assess how effective cleaning was on a paediatric CICU ward by use of an audit re-audit method, assessed against a 5-component bundle. This assessment of the reality of hospital cleaning would identify if and where failures were occurring, how this differed between staff group, and how best to design and implement a training bundle to improve cleaning as a whole. The following areas were considered during the study;

1. How well is cleaning being undertaken in the clinical environment?
2. How do different staff groups clean, and is there a difference in competencies between nurses and cleaners?
3. Assessed against a 5-point bundle created for this study, how do cleaners and nurses score? What areas are they failing in?
4. Can a small, staff-specific training scheme, based on these results, be effective in improving cleaning compliance?

The results from this chapter were published in Infection Prevention in Practise: Does Size Matter? The Impact of a Small but Targeted Cleaning Training Intervention Within a Paediatric Ward. [200]

7.3 METHODS

7.3.1 AUDIT BUNDLE CRITERIA

To assess all cleaning undertaken during the observations, an evidence-audit based tool was developed in order to create an audit standard. Cleaning assessments were based on 5 bundle-style components to judge each cleaning opportunity and generate a score, out of a possible 5, in which every component has equal weight. Bundle style interventions have been proven effective for delivering training in the clinical setting [409]. The following criteria fulfilled the bundle;

The 5-component cleaning bundle

1. **Has the entire surface been cleaned?**
This ensures all components of a surface are cleaned, including the undersides of objects.
2. **Was an 's' shape motion with overlap used?**
This motion is proven to clean the most effectively and ensure contamination is not dragged across a previously cleaned area.
3. **Was one wipe used for each surface (or until dirty or soiled)?**
The size of the surface must be assessed and the amount of wipes used as appropriate.
4. **Was the wipe folded out entirely?**
Folding out the wipe allows maximum surface area for cleaning, and for the capillary action of the wipes to work as per manufacturer design.
5. **Was the correct wipe used?**
Correct wipes or cloth was determined as per the local cleaning protocol. For cleaners, cloths and chlorine-based cleaning agent are to be used for surface cleaning. For nurses, alcohol wipes are to be used for IV tray cleaning, and Clinell Universal for all other surface cleaning. Paper towels or chlorhexidine cannula wipes are not to be used for surface cleaning.

Figure 7.1 The 5 components required for effective cleaning, used to generate a score for all cleaning observations.

The bundle components were chosen by assessment of the literature, and by consultation of GOSH IPC:

Bundle component 1

This component was determined based on the work by Sattar and Maillard [407], where it is shown that failure to full decontaminate a surface means other surfaces an easily be recontaminated. By failing to clean the entire surface, it cannot be classified as safe.

Bundle component 2

The ‘s’ shape motion with overlap is a proven method for ensuring the surface in cleaned in a manner that doesn’t recontaminate areas of the surface with a dirty cloth [410]. The ‘s’ motion makes sure the entire surface has been cleaned and soiling has not been pulled across previously cleaned sections [410].

Bundle component 3

This component follows evidence showing how a cleaning cloth or impregnated wipes have a limited volume of cleaning agent, and a limited surface area they can properly clean before there is risk of poor cleaning or contaminating a surface with a dirty cloth. As the cloth is use, the wipe becomes dry and contaminated, and must be disposed of and replaced with a new wipe [407, 408, 410].

Bundle component 4

This component ensures the wipe is being used to maximum effect. Folding out the entire wipe, as per the manufacturer instructions, allows the wipe to work as

designed. This will ensure organisms are captured properly and not released back onto a clean surface [407].

Bundle component 5

Different wipes are to be used under different circumstances for different types of surface, as per local cleaning policy. This component follows evidence in which using the incorrect cleaning device or cleaning agent can allow the transfer of organisms, and how different cleaning agents are more effective against different organisms [407, 408].

7.3.2 AUDIT OBSERVATIONS

Observations were undertaken in a paediatric 4-bedded bay within a cardiac intensive care unit (CICU). The observations were undertaken for 50 hours, assessing all healthcare staff that cleaned surfaces within the bed space environment. Patients and visitors were not included within the study, as this was assessment of healthcare workers only. Staff were broken down into; doctors, nurses, cleaners and others. The others category consisted of; housekeepers, physiotherapists, healthcare assistants and x-ray technicians.

To assess the cleaning, observations were undertaken by assessing all cleaning undertaken against the 5-component bundle shown in figure 7.1. How the staff cleaned and what they cleaned with was recorded. The observations were undertaken by two staff members using the same scoring criteria to ensure the results were consistent. They were both trained in cleaning, and were familiar with the bundle. In order to account for the Hawthorne effect, in which a person may modify their behaviour in response to being watched, the two staff members undertook the observations at the ends of the 4-bed bay which allowed easy observation of the bay without impeding staff cleaning or providing patient care. This would also ensure they were not in the way of clinical practise or intruding within the bed spaces. The observations were undertaken over 15 days in 1-3 hour periods, in which a range of times and days were observed, in order to account for the variation in ward activity between days and nights, or afternoons and evenings, capturing morning handovers, movement of patients and discharges. When completing the audit, the following staff cleaning roles were observed;

Table 7.1 Cleaning responsibilities for nurses and cleaners

	Cleaning Responsibilities		Cleaning Responsibilities
Nurses	IV tray	Cleaners	Floors
	Patient bed tables		Walls
	Nurses station keyboard and mouse		General wider environment
	Nurses station surfaces		Windows
	Equipment trolley top surface		Non-clinical surfaces post-discharge

7.3.3 Development of the education intervention

As the cleaning responsibilities varied between staff group, and the level of understanding of infection prevention and control varied, it was critical to build a training package that could target nurses and cleaners differently. Nurses and cleaners were selected as the focus for the intervention as the other observed groups of staff had no or few recorded cleaning observations. Doctors, physiotherapists, healthcare assistants and x-ray technicians had no recorded cleaning observations, and housekeepers only had N=4 cleaning observations before the intervention and N=5 post-intervention. The initial observations revealed nurses required more training in bundle components 1 and 2 (cleaning the entire surface, using an ‘s’ shape) and cleaners required from training in components 2 and 3 (using an ‘s’ shape, using one wipe per surface, or until dirty or

soiled). By streamlining the training and putting extra emphasis on the areas that required more improvement, it would, hopefully, allow the largest amount of improvement over a limited time. As nurses had a stronger background in Infection Prevention and Control, this could be used as an important baseline for additional training. For cleaners, it was important to give an overview of Infection Control basics, with emphasis on how their role keeps patients safe, and how this has an impact. Resources for training were developed. Design and printing, but not content, were provided by GAMA (GAMA healthcare, Watford, UK). The resources consisted of flashcards focusing on the 5 components of the bundle, reminder cards, and leaflets;

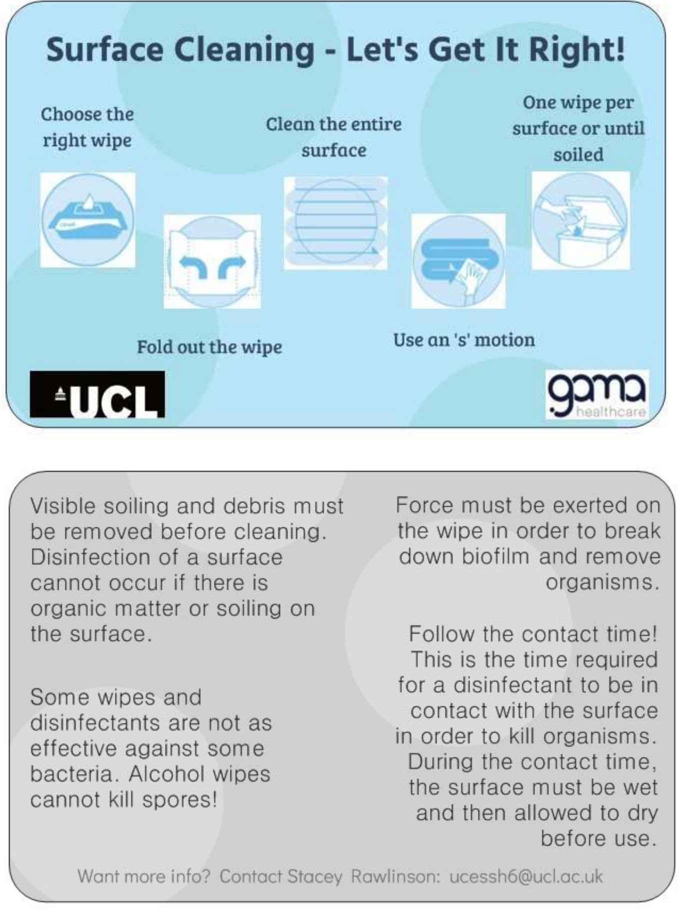


Figure 7.2 Training cards designed to fit in standard NHS badge lanyards front (above) and back (below) handed out during training.

Choosing the right wipe

Detergents

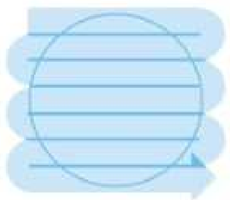
Detergents are critical to break down and remove dirt and organic matter prior to disinfection. This allows the disinfectant to make direct contact with microorganisms to ensure they are killed. However, detergent wipes alone do not destroy microorganisms. They pose the risk of picking up bacteria and transferring them to other surfaces.

Disinfectants

When cleaning surfaces in a hospital, you should always choose an effective disinfectant wipe to ensure that harmful microorganisms are killed.

Folding out the wipe

The fibres of the wipe are designed and woven in a way that easily picks up bacteria from the surface. This design can only work well when the wipe is folded out fully and used flat against the surface. This will also ensure surface gets the right dose of product to be effective.



Cleaning the entire surface

Harmful microorganisms can be present on any part of an object. Contamination on just one part of an item can be transferred along to other components, therefore all parts of the object should be considered potentially contaminated and effectively disinfected.

One wipe per surface

Disinfectants are not 'self-cleaning' and can become contaminated by bacteria. Detergents are even more susceptible. Using contaminated wipes or cleaning agents can spread bacteria between surfaces. Only one wipe should be used for each surface, and it should be disposed of when it becomes soiled or dry.

'S' shaped motion

Wipe the surface from top to bottom, clean to dirty, using an 'S' shape motion taking care to cover the entire surface without going over the same area twice. This ensures the entire surface is comes into contact with the wipe and that microorganisms are not spread back over areas that have already been disinfected.

Cleaning is critical to patient safety. The steps shown here should be followed carefully to ensure adequate cleaning.

Key notes

Visible soiling and debris must be removed before cleaning. Disinfection of a surface cannot occur if there is organic matter or soiling on the surface.

Know your enemy – all bacteria are not created equal! Some wipes and disinfectants are not as effective against some bacteria, for example, alcohol wipes will not kill some spores.

Use the force! Appropriate pressure must be exerted on the wipe while cleaning to break up dirt and organisms that may be adhered to the surfaces.

Follow the contact time - this is the time needed for a disinfectant to be in contact with bacteria to kill it. Bacteria are only killed when the surface is wet so it's important when using a disinfectant to let the surface air dry.

1. Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. Curr Opin Infect Dis. 2013;26(4):338-44.

JBN190546

in collaboration with

Figure 7.3 Detailed leaflet explaining each component of the training bundle.

7.3.4 DELIVERY OF THE EDUCATION INTERVENTION

The education intervention was delivered with the following components:

1. **Introduction to Cleaning**

(How microorganisms live on surfaces, why cleaning is important to prevent transmission of these organisms and protect patients)

2. **What are the components of cleaning?**

(The 5 bundle components with flashcards S1)

3. **Practical demonstration**

(Cleaning of a surface)

4. **Provision of handouts**

(Handout S2 given to staff to reinforce all training components, with reminder cards S1)

Delivering the education intervention in a practical format has been shown to be effective in improving IPC knowledge in domestic staff [378]. The design of the training was the same for both staff groups, and the practical sessions delivered were identical. Where the pre-audit observational revealed poorer compliance in some areas of the bundle (component 1 and 2 for nurses, and components 2 and 3 for cleaners) the design of the training ensured these were covered with sufficient detail in both the why and how of cleaning, with practical demonstration.

7.3.5 STATISTICAL ANALYSIS

Analysis of scores was undertaken using an unpaired t-test. The individual bundle components were assessed using one-way ANOVA. All statistical analysis was undertaken in OriginLab2018.

7.4 RESULTS

7.4.1 PRE-INTERVENTION AUDIT

The pre-intervention audit consisted of assessing cleaning, using the 5-point bundle (figure 7.1), before any training or intervention was undertaken. Here, a total of 26 cleaning opportunities carried out by nurses and 92 by cleaners were observed over 50 hours. As so few staff from the others category were observed cleaning, they were not considered for data analysis. Doctors, physiotherapists, healthcare assistants and x-ray technicians had no recorded cleaning observations, and housekeepers only had N=4 cleaning observations before the intervention and N=5 post-intervention. Before any training intervention, of a possible 5, nurses scored an average (mean) of 2.4 across all (N= 26) observation, and cleaners scored an average (mean) of 2.5 across all (N= 88) observations, as shown in figure 7.4.

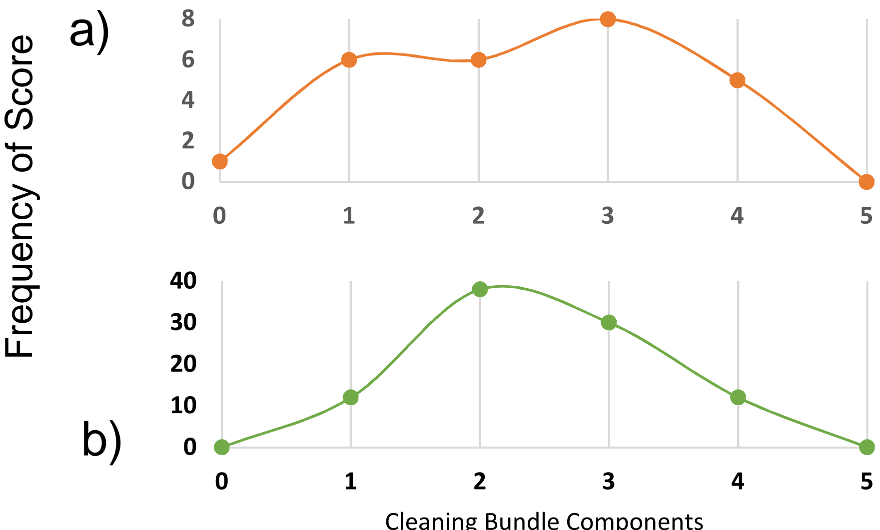


Figure 7.4 Frequency of scores achieved for a) nurses and b) cleaners prior to the training intervention for each cleaning opportunity.

7.4.2 EDUCATION INTERVENTION AND POST-INTERVENTION AUDIT

During the education intervention, an effort was made to train as many of the ward staff as possible. A total of 103 (69%) of the nurses and 5 (100%) of the cleaning staff received the training. A total of 38 training leaflets (figure 7.2) and 118 reminder badge cards were given out (figure 7.3). A total of 33 cleaning observations were recorded post-intervention for nurses, and 88 for cleaners. After completing the training, both nurses (P= .004) and cleaners (P= .0003) showed significant improvements in compliance and bundle scores, improving from 2.4 and 2.5, to 3.3 and 2.9, respectively (figure 7.5). This produced a total improvement in compliance of 18% for nurses and 8% for cleaners.

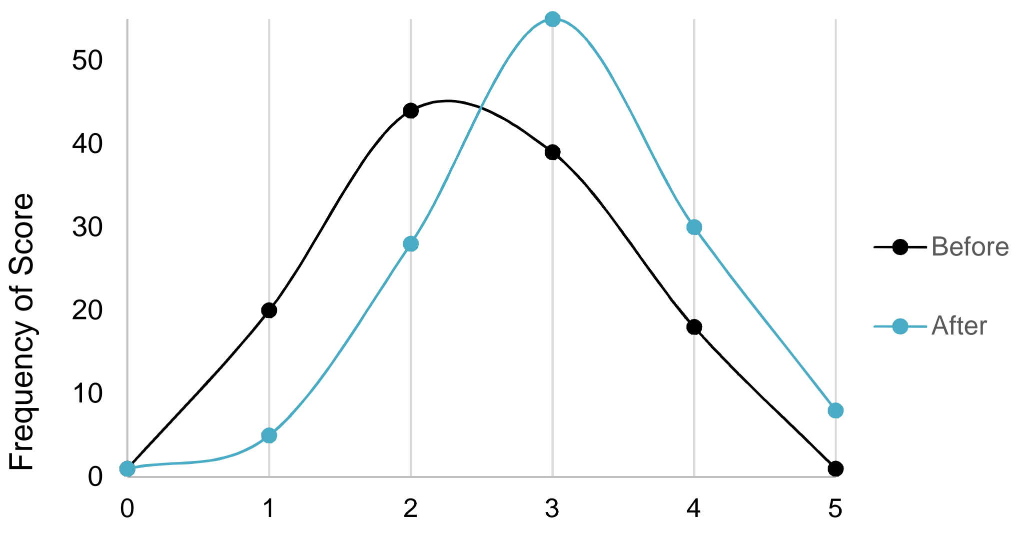


Figure 7.5 Cleaning bundle scores achieved, for nurses and cleaners, before and after implementation of the training intervention.

Compliance improvements for individual surface cleaning varied, and is shown in figures76.6 and 7.7, for some surfaces that are only cleaned by nurses or only cleaned by cleaners, so that the differences in cleaning responsibilities and impact on the surfaces before and after the training might be represented. Before

the training intervention, the bin had 9 individual observations and 11 after the training. For cleaners, the floor had 10 before training, and 8 after. The soap and hand gel dispensers had 10 before and 8 after, and the chairs had 7 before and 9 after. For nurses, the IV trays had 18 individual cleaning observations before the training, and 25 after. The other surfaces category for nurses had 9 before and 10 after.

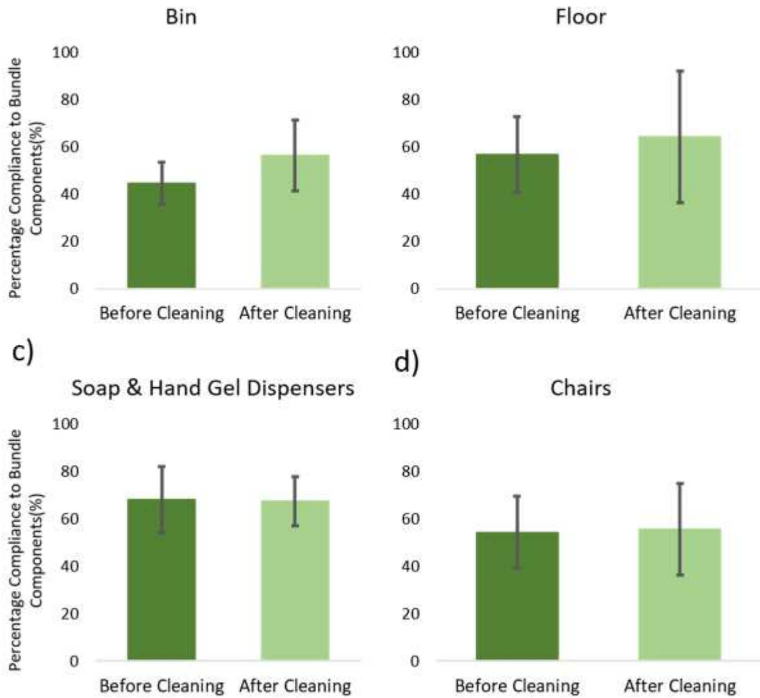


Figure 7.6 Average (mean) Compliance to bundle components for cleaning of individual surfaces for Cleaners before and after the training intervention of ; a) clinical and domestic waste bins, b) floors, c) hand soap and hand gel dispensers, d) staff, visitor and patient chairs.

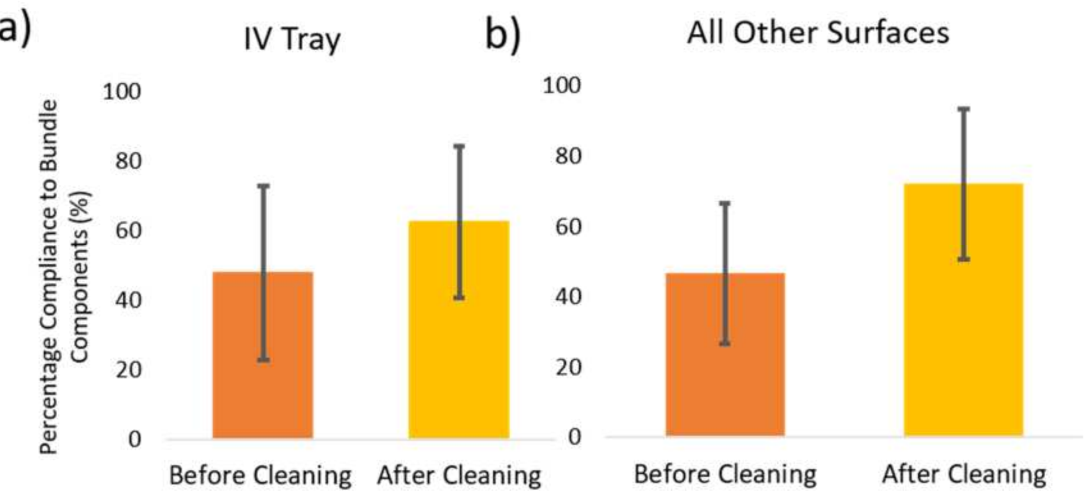


Figure 7.7 Average (mean) compliance to bundle components for cleaning of a) IV trays and b) all other surfaces for nurses before and after the training intervention.

It is unsurprising that different surfaces would vary in how well they have been cleaned, due to personal perception of risk and physical size and shape. As expected, compliance between cleaning different surfaces varied. Cleaners had an improvement of 11.92% for cleaning bins and 7.5% for floors, though to no statistical significance (P >.01). Very little differences were seen for chairs, at 1.27%. Soap and hand gel dispensers had poorer compliance, at a reduction in compliance of 0.5% after training. For nurses, IV tray cleaning improved by 14.62% (P= .0475) and all other surfaces had 25.33% (P= .0168) improvement.

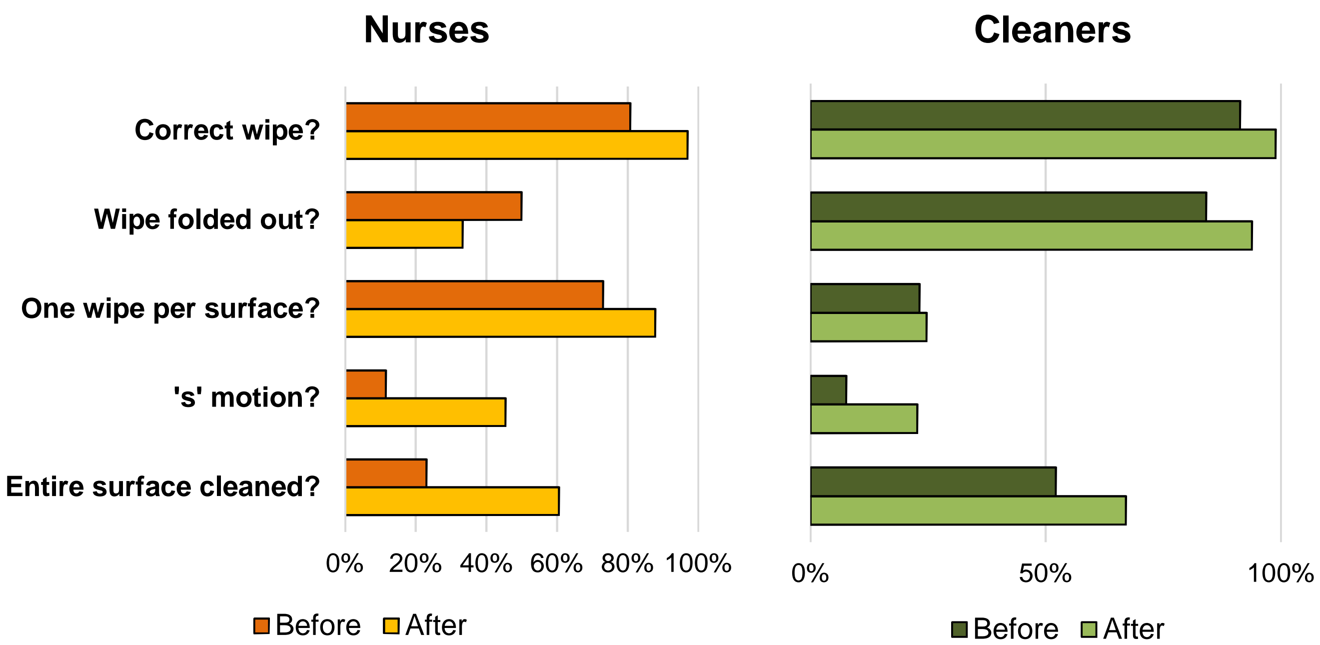


Figure 7.8 Average (mean) compliance to individual cleaning bundle component for nurses and cleaners before and after the training intervention.

When considering individual compliance to the different bundle components and the variation between staff groups, it is clear that compliance varies between staff group both before and after the training. Compliance varied between nurses with a range of 0-80%, and cleaners by 20-80%. The pre-intervention audit revealed nurses required improvement in cleaning the entire surface and using the 's' motion (23% and 12% compliance respectively) and cleaners required improvement with using 's' motion and a single wipe for each surface (8% and 23% compliance). Following the training, focusing on these specific areas, overall improvement was found for nurses (P= .004) and cleaners (P= .0003), as well as a change in compliance for each bundle component. All bundle components for both staff groups showed improvement following training, except for a reduction (-17%) in compliance for folding out the wipe for nurses.

Following the training intervention, nurses had the most improvement (18% overall) in cleaning the entire surface (P= .003) and using an 's' motion with 15% improvement (P= .004), which were the focus of the training. Cleaners had the most improvement in the same two bundle components, one of which was the focus of the training intervention. This had a small but statistically significant improvement of 15% (P=.004). Cleaners had an 8% improvement overall. Despite being a target for improvement, using one wipe per surface had insignificant improvement (P= .855) at just 2%.

7.5 DISCUSSION

This study sought to assess cleaning compliance within a paediatric ward to a specifically designed audit criteria, with staff-group specific targeted training based on these audit observations, and to assess the efficacy of this training intervention with the same audit criteria. Overall, the study showed a modest improvement (P<.0001) in cleaning compliance as assessed by the bundle. Prior to training, 2.4 of 5 bundle components were adhered to. Following the training, this improved to 3. For nurses, a score of 2.4 improved to 3.3 (P=.004) following training, and cleaners improved from 2.5 to 2.9 (P=.0003).

The importance of cleaning in the healthcare settings is well-recognised. Many studies have shown how a wide range of pathogens can be recovered from surfaces as proof of poor cleaning [139, 411, 412]. However, as previously explored in chapter 1 and 6, surfaces are often neglected, particularly surfaces that are not classified as 'high-touch' or near patient surfaces. Failures in cleaning in clinical settings are an ongoing issue, and are documented across the world [4, 413]. Cleaning is the first line of defence against HCAIs, and effective cleaning is associated with lower incidence of HCAI [208]. Invasive medical procedures that break the skin leave patients extremely vulnerable to HCAI, and cleaning can help mitigate the risk from environmental contamination.

As antibiotic resistance is on the rise, and antimicrobial stewardship is becoming more important [414], cleaning to prevent HCAI acquisition and therefore the use of antimicrobials can be an important step in reducing the dissemination of antimicrobial resistance; prevention is better than cure.

7.5.1 CLEANERS – THE UNDERAPPRECIATED BACKBONE OF THE HOSPITAL

The most obvious heroes of the clinical environment are often the doctors and nurses. Cleaners play such an important role in keeping patients and staff safe, and yet cleaning is considered a low-skilled job. The reality is that without cleaners, healthcare premises could not function. Despite this, cleaners are often forgotten. They feel unappreciated, not respected, and this is reflected in their wages and job outlook. Studies have shown that cleaners do not feel valued, or considered as part of the hospital team [415]. Some domestic workers are left feeling as though their roles are not important, and they are not given adequate support [196, 415]. Feeling undervalued and unsupported, in any job role, can undoubtedly damage morale and lead to lower compliance.

7.5.2 TRAINING – A COMPLICATED ISSUE!

Training is key to improving cleaning efficacy and compliance. Effective communication, delivery and frequency of training can improve cleaning efficacy. Failures in cleaning are often due to lack of competency, caused by poor training. Cleaning training is an underestimated issue. Quality, frequency, type and content of cleaning training varies between hospitals [191, 201]. However, despite training being so critical, there is no standard cleaning training protocol. Often, for cleaners, training is not delivered as a distinct specific component of induction, and simply given by their peers as they complete their job. This undoubtedly leads to reduced quality of training as this information is passed down, as key elements are lost by attrition over time. During the implementation of the training intervention, it was clear, even within the same staff groups, the level and quality of training previously

given varied. This disparity in training was well-documented in a review of cleaning across hospitals, which showed this wide variation in cleaning training quality and type [191]. An international survey revealed only 46% of staff were given yearly cleaning training, 15% twice-yearly, and 20% sporadically [201].

Compliance is also measured in different ways. Within the UK, under the Health and Social Care Act, there is a requirement for clinical environments to be safe. However, this is difficult to define as there are no standards, legislation or consensus about what constitutes as 'safe' concerning the level of surface contamination. Currently, there is some guidance available from the National Specifications for Cleanliness in the NHS [337] on general monitoring of the hospital environment, in which surfaces are assessed by visible audit only, and no microbiological screening is indicated. Therefore, good cleaning may be determined as surfaces simply looking clean, which gives no bearing on the microbiological safety of a surface. Some hospitals go further, and use other methods to assess their cleaning, such as auditing the removal of UV markers following cleaning, or the use of ATP monitoring. The advantages and disadvantages of these methods have been explored in chapter 2, though neither are comparable to true microbiological sampling. None of these are mandated, however, how a cleaning teams' compliance is measured, if at all, is down to local policy and opinions.

7.5.3 DESIGNING TRAINING - ONE SIZE DOES NOT FIT ALL

With a lack of guidance overall, designing effective training is a difficult task. It is also important to consider the needs of those receiving the training. In this study, cleaners and nurses were assessed separately. With different levels of training and

IPC knowledge, it is unsurprising that their compliance to individual bundle components was found to differ. Figure 7.4 shows how the compliance varied between staff group and bundle component. Nurses had the lowest compliance in cleaning the entire surface and using the 's' motion (23% and 12% compliance respectively). Cleaners had lower compliance with using 's' motion and a single wipe for each surface (8% and 23% compliance), as shown in figure 7.8. As such, designing training based on these findings could potentially increase the impact of a training intervention. As the intervention was designed to be small scale and require few resources, enhancement by producing specific targeted training for each staff group could improve its impact. With limited time available, re-training of all components of cleaning and IPC basics were not possible. By knowing the specific areas that required improvement, additional time could be allocated for the bundle components that required the most improvement. Additionally, training must be different as nurses and cleaners will have different basic training which can be expanded on to add clarity and understanding. For nurses, the training could provide more detail on pathogen transmission and IPC. For cleaners, very basic details on transmission were given, and their training focused on the mechanics of the wipes, as well as the specific importance of their role, and how they were so very important in preventing patient from contracting potentially deadly HCAI.

Training to clean should never just be an instruction on how to physically clean a surface. Lack of understanding has led to cleaning failures. Throughout the observation study, many of the failures were due to basic lack of understanding on the fundamentals of cleaning, which highlights a specific issue in training, such as the

lack of understanding on why cleaning motion or action, or using an ‘s’ shaped motion would be an important step in cleaning. The lack of understanding of how poor technique may spread organisms on a surface or cross contaminate cleaner parts of a surface came from the fundamental gap in training on why cleaning should be undertaken a certain way in relation to the basic knowledge of IPC.

With some additional explanation as to why the different aspects of the bundle are so important, this could allow staff to make informed choices when cleaning. Instead of simply remembering a set of motions, their new understanding of cleaning would allow them to clean according to best IPC practise.

Additionally, personal perception plays a role in cleaning efficacy as discussed in sections 6.4.2.2 and 6.5.2; surfaces classified as easier to clean had the greatest increase in CFUs following cleaning, (34.13%) when compared with surfaces classified as difficult to clean (18.31%). When cleaning, staff members make subconscious assessments of their surfaces, often with poor or incorrect training. They will determine how easy the surface is to clean and the risk they deem this surface to be to the patient, all of which will impact their cleaning [317]. These choices made with poor IPC knowledge could lead to poor cleaning practice. These impacts have been discussed further in chapter 6, though it is important to consider these as a target when delivering the cleaning training.

7.5.4 IMPLEMENTING EDUCATION-BASED CLEANING TRAINING INTERVENTIONS

This study sought to produce and implement training that was feasible for the busy clinical ward. Ideally, training would be delivered regularly, as a separate event with reserved time and a dedicated space. However, in a busy clinical environment

with ever-increased patient load and staffing issues, this is not possible. Therefore, producing a large, detailed training intervention would not work in this context, despite the fact that large multi-faceted training interventions have been shown to work in other settings [159, 197, 198]. Therefore, the focus was on delivering the training as quickly as possible, with the ability to be delivered bedside, be adaptable, and to be staff-group specific. The CICU setting in which the training was delivered only reiterated the importance of 'portable' training interventions. Within CICU, nurses were unable to leave their patients' bed spaces. Here, the training could be delivered bedside, or at a strategic point within a 4-bed bay to capture any staff within the area. This proved effective as a good proportion of a busy working cohort received the training due to this format, and even within the CICU setting in which access to bed spaces could be limited due to a wide variety of factors, such as medical emergency, the training was delivered to 69% of the ward nurses. This 'on the go' factor was less important for cleaning staff, who were able to take a moment aside from their role, in which 100% of staff were captured.

Other education-based cleaning training intervention studies have been completed in the clinical environment and have shown to have improvement in cleaning compliance. For this study, improvements were documented as increased compliance to the 5-point bundle by observation of cleaning. Assessing improvements following cleaning training varied between studies, from observations [200] to UV markers [159], to evaluation of change in HCAI rate [198], to a combination of these, including staff attitude changes and surface bioburden of high-touch surfaces [197]. Evaluation of HCAI rate is the gold standard of viewing how

much real impact a cleaning training intervention has had. While audit re-audit observations can assess if cleaning compliance has improved and that cleaning is of a better standard then before any intervention is delivered, more comprehensive methods are needed in order to assess if the cleaning is truly improved in terms of reducing HCAI. It is the assumption that improvements in cleaning will relate to improvements in reducing environmental bioburden, usually assessed by CFU or ATP systems. It is again assumed that reduction of CFU or ATP will have a positive correlation in reducing HCAI. General CFU or ATP are indicators of cleanliness and not of specific risk. Assessing HCAI trends in parallel with cleaning training interventions will give the true answer to the question; is this training effective? A 12 month study by Allen *et al.* 2018 implemented a cleaning training intervention and assessed the results against HCAI data for MRSA, VRE, *K. pneumonia* and *C. difficile,* though found no significant difference in change of HCAI incidence [198]. A longer study across 11 hospitals with a multimodal bundle-style cleaning training intervention found that the incidence of VRE reduced N= 230 cases before intervention and N= 50 after intervention, but that *S. aureus* bacteremia and *C. difficile* infection did not change significantly. Both studies reported a positive change in cleaning compliance. And while bigger studies are not always better, and there needs to be a plateau of time and money invested versus training interventions outcomes, this format of HCAI-incidence based assessment give a deeper analysis of how effective a training intervention has been. Such deeper analyses give more ammunition in the fight for better cleaning training and investment in large multifaceted training, as one study found that introduction of just one more cleaning

member of staff gave a 26.6% reduction of *S. aureus* acquisition by patients, saving the hospital between £30,000-70,000 [132]. In comparison, implementation of a multi-modal cleaning bundle within a 400-bed hospital with a 3-month pre-intervention and a 6-month post intervention stage had a cost of approximately £10,600.

While the end goal of cleaning training is to reduce overall HCAI rates, monitoring impacts of training interventions themselves can be a time and resource-consuming process, which would not be applicable to all healthcare environments.

Due to differences in method, study size and audit criteria, different training interventions cannot be directly compared, though the simple 5-point bundle intervention was effective when weighed against larger, multi-faceted interventions. One large-scale intervention had increased cleaning of bathroom touch points from 55% to 75%, and in bedrooms improvement from 64% to 86% compliance assessed by UV marker removal [197]. Another large intervention revealed improvement by 34.3% assessed again with UV marker removal [198]. While this study did not assess specific surface cleaning improvement due to the nature of the ward making surface sampling difficult, compliance improved by 18% for nurses and 8% for cleaners, which would hopefully translate to increased and more effective removal of surface bioburden.

7.6 CONCLUSIONS

This work sought to evaluate the impact of a small but targeted education-based cleaning training intervention, with a staff-specific intervention implemented by assessment on areas of failure by a 5-point bundle audit. The training proved effective. Overall compliance to bundle components was a 2.5 score of a possible 5. A targeted training intervention was found to have a small but significant (P <.0001) improvement to 3.0 overall in cleaning compliance, in both nurses and cleaners. Due to differences in how successes of an intervention were assessed, this intervention could not be compared to its larger-scale counterparts, thought it can be said the improvement was modest when compared to other interventions. This chapter identified how training has many factors, and training must be carefully designed and delivered in order to have maximum possible impact. Procedure-based training has less impact than explaining why different components are important for cleaning efficacy, instead of just the physical action required.

Cleaning and domestic services have often been considered low-skilled jobs, and their wages reflect this perception. It has been suggested that cleaners don't feel respected or valued. While the overall perception of cleaners and their role cannot be changed quickly, how cleaners value their job and see their role was a target for training. It is critical they know just how instrumental they are in the running of the hospital. They also must be provided with the information they need in order to do their job best. It must be accepted that, like hand hygiene, cleaning compliance will never be 100% [198, 416]. In the events where cleaning teams cannot fulfil their duties for any reason (time or resourcing restraints) we must arm them with the

knowledge of what surfaces are of most value to clean, when value is determined as risk to patient. However, when cleaners are in charge of cleaning an entire ward, knowing what surfaces within this space may or may not be most critical can be difficult to determine. Building knowledge of how contamination or a surrogate for contamination, moves around a ward environment can help inform cleaning training interventions to give cleaners insight into what is happening on their surfaces.

Chapter 8 Validating a Surrogate Material to Track Infection Movement in the Clinical Space

8.1 Introduction

The use of a 'surrogate' or replacement material for tracking infection and identifying potential reservoirs and to assess cleaning efficacy is not a new concept. Often, this is done by the use of adenosine tri-phosphate (ATP) sampling, in which swab-based sampling devices measure the amount of ATP on a surface. More ATP in the swab leads to an increased bioluminescence reaction, called relative light units (RLU). Manufacturers of these devices claim RLU can generally relate to CFU, though these claims are fundamentally flawed for several reasons, such as the limitation of the swabs on visibility dirty surfaces. Another option is ultraviolet (UV) reactive powders and liquids, which can be viewed only with UV torches. This can show how quickly a material can spread between surfaces and hands, and give cleaning teams an idea of how effective their cleaning efforts have been. While these methods are fast and easy, they are not without large limitations. These methods do not provide accurate quantification, and cannot track movement throughout the environment. Using cauliflower mosaic virus (CaMV) as a surrogate is an accurate way of quantifying spread and deposition, and movement throughout the clinical environment.

Cauliflower mosaic virus is a double stranded plant pathogen that is transmitted by aphids, causing a wide variety of symptoms dependant on species of plant and environmental conditions [417]. It has a total length of 8024 nucleotides [418]. Cauliflower mosaic virus was chosen for this study as it has proven potential

as a non-infectious surrogate marker for environmental infection transmission, used previously in two studies [419, 420]. Even with ingestion of CaMV-contaminated vegetables, there are no implications for human safety. Inoculation of a pathogen onto a surface can be mimicked with the DNA marker, and subsequent transmission throughout the surface environment tracked, while remaining a safe, non-infectious and appropriate method for tracking infection movement, even in sensitive areas such as paediatric intensive care units (PICU). It also fulfils the requirement for easy quantification and recovery from surfaces. Tracking this surrogate marker provides greater accuracy and applications in comparison to other methods currently available, such as the use of UV powder to simulate spread of infection.

In 1985, the first amplifications of target DNA using polymerase chain reaction (PCR) were achieved with *Escherichia coli* DNA polymerase [421]. In 1988, amplifications were achieved using *Taq* DNA polymerase [422]. PCR is the process in which target DNA is cycled through heating and cooling stages, allowing the amplification of even the smallest amounts of target DNA, facilitated by an enzyme reaction. A long timeline of discovery and method adaptation (figure 8.1) has allowed PCR to become widely available.

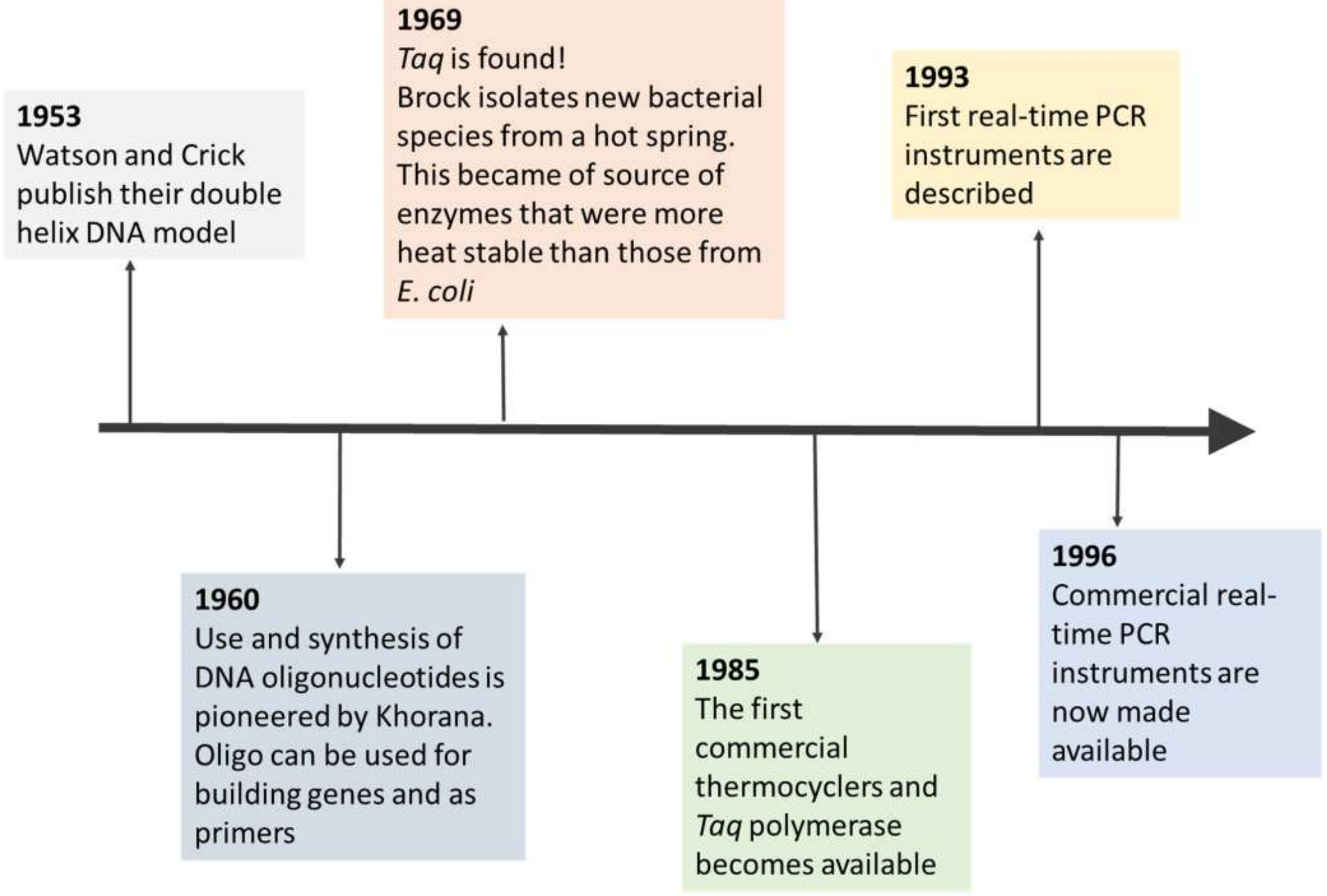


Figure 8.1 Basic timeline of PCR discovery and development

The DNA markers can be recovered from the swab samples and processed using qPCR (quantitative polymerase chain reaction). qPCR is an advancement on traditional PCR in which a fluorescence reaction is used. When the fluorescence of the target reaches above the background signal, a cycle threshold (Ct) is reached. Lower Ct values are associated with greater concentrations of the target DNA. These Ct values can be used for quantification following set-up of a standard curve, such as providing results in number of viral copies within a sample. This quantification of a specific target makes this method useful for a wide array of microbiological applications, as well as for a hospital ward tracking study.

Two other studies have used CaMV-based DNA markers as a surrogate material. This chapter expands on previous studies in several ways, including the removal of the ampicillin resistance gene (AMP^r) to ensure the inoculums have no potential resistance transferred to hospital organisms, increase in number of inoculations from one surface to three surfaces to see movement and interactions, and size of sampling study, of which this thesis expands upon by sampling an entire ward. Previous studies also did not quantify recovery as the method design only allowed for presence-absence testing of the oligonucleotide and sampled a very small range of environmental surfaces (12 sites, 10 sites and 32 sites). This thesis used complex method design so recovery could be quantified, interactions between three separate markers could be assessed, and sampled sites across the entire ward (44 sites daily).

Knowing where contaminated surfaces are within a clinical environment is only some of the picture. In order to build truly evidence-based sampling and cleaning training protocols the transmission of organisms around the environment must be shown. Transmission studies can identify key moments that microbiological sampling cannot. By designing a surrogate marker than is safe and can be used in any healthcare facility, both paediatric and adult, can inform and assist in the development of targeted cleaning protocols. Identifying surfaces that lead to significant transmission events can help IPC teams 'cut off' the chain of transmission. Additionally, marker transmission can not only be an assessment of cleaning, but of ward design efficacy and insight into how if contact precautious are being adhered

to. This chapter designs and validates a surrogate marker than can be used across all healthcare facilities.

8.2 RESEARCH AIMS

This chapter aims to use an oligonucleotide based on a plant pathogen as a surrogate for infection.

1. Develop an oligonucleotide that would move around the environment as a surrogate for a pathogen, while remaining safe to release in a paediatric clinical environment.
2. Develop appropriate and optimised sampling methodologies for recovering the oligonucleotide from the surface environment.
3. Validate the use of this oligonucleotide as a surrogate marker, assessing how the marker persists on a surface, can be cleaned, is transferred by hands, and how it is removed following hand hygiene.

8.3 METHODS

8.3.1 OLIGONUCLEOTIDE DESIGN

Three 400 base pair (BP) strands, that had no overlap and had appropriate primer pairs as assessed by Primer3plsus software were selected at random from the CaMV sequence by downloading the entire Cauliflower Mosaic Virus sequence (accession number: KF357594) and sent for synthesis (IDT, Belgium). These separate strands would be used for inoculating three separate sites within the hospital for tracking. Three markers were chosen as an appropriate number to make sampling recovery viable across an entire hospital ward, and to see interactions between the three markers from three distinct areas within the hospital ward; an isolation room, a treatment room and the reception (detailed in section 9.3.1). Following qPCR analysis, these separate strands would give distinct results using separate primer pairs, meaning they could be identified separately, and both origin from an inoculation site and concentration could be determined.

Table 8.1 Sequence of three synthesised oligonucleotides to be used as markers for surrogate infection, with primer pairs and highlighted annealing sites and amplicon sizes.

Marker	Sequence (at 400 BP each)	Forward Primer	Reverse Primer	Amplicon size
1	GGTATCAGAGCCATGAATAGGTCTAAGACCATAACTCAAG AGGGTAAAACCTCATCAAAATACCAAAGAGTTCTTAACTCT AAAGATAAAAGATCTTTCAAGATCGAAAACTAGTTCCCTCA CACCGGTGACCGATAGGTTTACCACCGTAAGGTTTCAGAAC AACATCGAATGCGTTTACGCCAACTTCGACTCTCAGCTCAA GTCGTCGTACGATGGTGATCTAAAAAGATCAAGAATCTAA GCCTTAAAAATCTTAGATGTTACGAAGCCTTCCTCAGGAAG TACCTTCTGGAACAATAAAATCTCTCTGAGAATAGTACTCTA TTGAGTATCCACAGAAAAAAATAATCTTCTGTGTTGAGATGG ATTTGTATCCAGAAGAAAACACCCAAAGCGAG	ACTAGTTCC CTCACACCG GT	CGTACGAC GACTTGAG CTGA	106
2	AATCCAGTACTAAAATCCAGATCTCCTAAAGTCCCTATAGAT CTTTGTGGTGAATATAAACCAGACACGAGACGACTAAACCT GGAGCCCAGACGCCGCTTGAAGCTAGAAGTACCGCTTAGG CAGGAGGCCGTTAGGGAAAAGATGCTAAGGCAGGGTTGG TTACGTTGACTCCCCCGTAGGTTTGGTTTAAATATCATGAA GTGGACGGAAGGAAGGAGGAAGACAAGGAAGGATAAGG TTGCAGGCCCTGTGTAAGGTAAGAAGATGGAAATTTGATA GAGGTACGCTACTATACTCATACTATACGCTAAGGGAATGC TTGTATTTACCCTATATACCCTAATAACCCCTTATCGATTTAA AGAAATAATCCGCATAAGCCCCCGCTTAAAAAATT	GACGCCGCT TGAAGCTAG AA	TTTAAGCG GGGGCTT ATGCG	304
3	ACATGTACAAGACGGAACTGGCGGATTTCCCAGGATATAT CAACCAGTACCTGTCAAAAATTCCCATCATTGGAGAAAAAG CGCTAACACGCTTTAGACATGAAGCCAATGGAACCAGCATC TACAGCTTAGGTTTTGCGGCGAAGATAGTCAAAGAAGAAC TATCTAAAATCTGCGACTTATCCAAGAAGCAGAAGAAGTTG AAGAAATTCAACAAGAAGTGCTGTAGCATCGGAGAAGCTT CAGTAGAATATGGATGCAAGAAGACATCCAAGAAGAAGTA TCATAAAAGATACAAGAAAAAATATAAGGCTTATAAACCTT ATAAGAAGAAGAAGAAATTCCGATCCGGAAAATACTTCAA GCCCAAAGAAAAGAAAGGCTCAAAGCAAAAGTATTG	TGTACAAGA CGGAACTGG CG	TGAGCCTT TCTTTTCTT TGGGC	383

Primers were assessed for GC content, and that no primer-dimers would be created. These primers were assessed in silico using the primer3plus software

(Untergasser, Germany). Three primer pairs for three separate cauliflower strands were chosen. These were cross-referenced in silico to ensure there would be no binding and interactions between the three different primers and strands of CaMV. An additional primer pair (PUC13_F CACGACGTTGTAAAACGAC, PUC13_R GGATAACAATTTCACACAGG) was also created using a commercial sequence for P-GEMT easy vector, in order to assess successful insertion of chosen sequences into the vector. PUC13 primers were based on the commercial sequence (Promega, UK) and synthesised (IDT, Belgium).

8.3.1.1 Resuspension, A-tailing and ligation

The oligonucleotides were shipped as a dry powder (IDT, Belgium). This required resuspension to a concentration of 10ng/µl. Oligonucleotides suspended in water or Tris EDTA (TE buffer) at a pH of 8 can be kept frozen and will remain stable for several years.

1. Upon delivery, the oligonucleotides were centrifuged at 3000xg for one minute to ensure all powder content collected in the bottom of the tube.
2. 50µl of TE buffer was added to each oligonucleotide.
3. Oligonucleotides were vortexed and incubated in a heat block for 20 minutes at 50°C.
4. Following incubation, the oligonucleotides were vortexed for 30 seconds and centrifuged at 3000xg for one minute. These were now ready for the A-tailing process.

8.3.1.2 LIGATION INTO pGEM-T

Oligonucleotides were shipped blunt-ended. The A-tailing protocol added the necessary 'A' overhangs to produce compatibility with the pGEM-T vector. The following contents were added into a 0.5ml microcentrifuge tube:

Table 8.2 Components added for the 'A' tailing of oligonucleotides

Components	Volume (µl)	Final amount
gBlocks Gene Fragments (10 ng/ µL)	5	50 ng
Taq polymerase (5 units/µL)	0.6	3 units
Taq polymerase buffer (10×, $MgCl_2$ 15mM)	1.5	1×, 1.5 mM
dATP (0.5 mM)	1.5	0.05 mM
Nuclease-free H_2O	6.4	-
Total Volume	15 µL	

The components were incubated using a heat block for 30 minutes at 70°C. This product was then used for the ligation step. The following components were added to 0.5ml tubes with low DNA-binding capacity:

Table 8.3 Components added for the ligation process of the oligonucleotides

Reagents	Reaction (µl)	Positive Control (µl)	Negative Control (µl)
2X Rapid Ligation Buffer	5	5	5
P-GEMT plasmid (50ng)	1	1	1
A-tailed oligonucleotide	1	-	-
Control Insert DNA	-	2	-
T4 DNA ligase (3 Weiss Units/µl)	1	1	1
Deionised water	2	1	3
final volume of:	10	10	10

pGEM-T vector, an artificially constructed plasmid, and control insert were centrifuged at 3000xg for 30 seconds to collect the contents at the bottom of the tube. 2X concentration ligation buffer was vigorously vortexed between uses. Reactions were mixed by pipetting, before incubating at 4°C overnight to produce the maximum number of transformants.

8.3.1.3 TRANSFORMATION INTO COMPETENT E. COLI CELLS

Following A-tailing and ligation, the oligonucleotides must be inserted into the plasmid. Transformations were undertaken using High Efficiency Competent (*E. coli*) Cells (Promega, UK). Each individual 400BP were inserted into a pGEM-T easy vector (Promega, UK) which has resistance to ampicillin. Ligation and transformations were completed using the Promega protocol. Insertion site was shown in figure 8.2. The following steps were undertaken for transformation:

1. Ligation reactions (contents in table 8.3) were centrifuged at 3000xg for 30 seconds.
2. 2µl of each reaction was added to sterile 1.5ml tubes on ice.
3. The *E. coli* Competent Cells were thawed in an ice bath for 5 minutes and mixed by flicking the tube gently.
4. 50µl of competent cells were aseptically transferred into the ligation reactions. 100µl of competent cells were added to the background tube (control tube).

5. Cells were heat-shocked for 50 seconds in a water bath set to 42°C, then placed in ice for 20 minutes.
6. 950µl SOC medium (Super Optimal broth with Catabolite Repression) was added to the ligations, and 950µl was added to background tube.
7. Reactions were incubated for 1.5 hours at 37°C with 150rpm shaking.
8. Luria Bertani (LB) agar (Invitrogen, UK) plates were prepared as per manufacturer instruction with the addition of 100µl ready-made solution of ampicillin (Sigma, UK) per 100µl broth added once cooled to approximately 55°C. Once plates were set, they were spread with 40µl blue-white select reagent (Sigma, UK) using sterile plastic disposable spreaders and allowed to dry.
9. Following incubation, 100µl of each reaction were plated out in duplicated onto LB agar plates with ampicillin at a concentration of 100mg/l.
10. 100µl of ligation reactions were inoculated onto LB/ampicillin plates with blue-white screening reagent, spread with a sterile l-shape spreader in a figure eight motion, and incubated overnight at 37°C (Classic incubator, LEEC, UK).
11. Following incubation, white colonies were selected and inoculated into 40ml LB broth with ampicillin, and allowed to grow in the shaker overnight at 37°C (Stuart, UK). This broth was now ready for the extraction process.

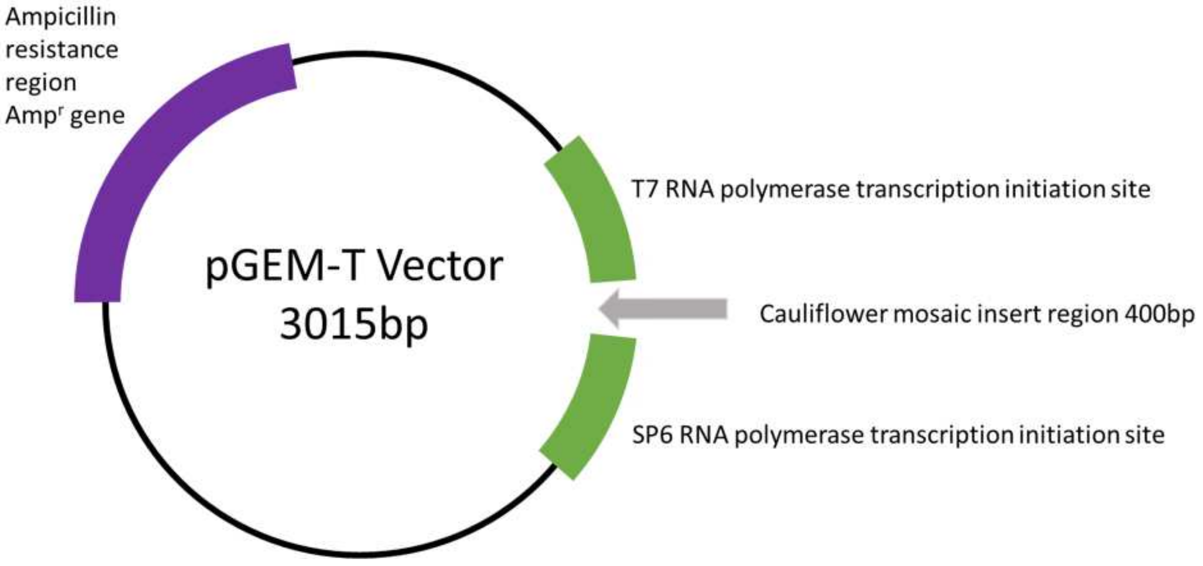


Figure 8.2 pGEM-T Easy vector with sequence reference points and insertion region.

8.3.1.4 Plasmid Extraction

Following successful insertion into the plasmid, the DNA required extraction. Extraction processes were undertaken in a separate lab to avoid environmental contamination with hardy plasmid DNA. Extractions were undertaken using the QIAGEN mini plasmid extraction kit, following the protocol as per manufacturers instruction, with a slight amendment during the elution stage, in which 50µl of buffer was used, allowed to stand for 5 minutes, and centrifuged for 1 minute at 13,000RPM. Another 50µl of buffer was added and centrifuged a second time to allow maximum DNA elution from the column. Prior to the washing stage, sample repeats were pooled into single columns to increase overall DNA yield, as losses were to be expected in the following manipulations to remove the ampicillin resistance.

Extracted DNA contents were immediately diluted by 1:10,000, and 1:10 dilutions. Concentrations were analysed using the Qubit flouorometric system (Thermofisher, UK). Following concentration analysis, insertion was confirmed using

p-GEMT primers (PUC13) and specific primers to sequence the cauliflower inserts only.

8.3.1.5 PLASMID LINEARISATION AND REMOVAL OF AMPICILLIN RESISTANCE

Plasmids are the major contributor to the spread of antibiotic resistance. It is important to consider the small yet potential risk plasmid-mediated resistance transfer could pose during this experiment. p-GEMT uses ampicillin resistance (provided by the AMPr gene) for user-friendly transformation and selection of cells with insert. In order to remove this risk, specific enzymes were used to cut out the ampicillin resistance from the plasmid prior to release in the clinical environment. Visualisation software revealed BspHI and AcuI commercial enzymes would cut the resistance, while leaving the 400BP cauliflower insert intact (figures 8.2 and 8.3).

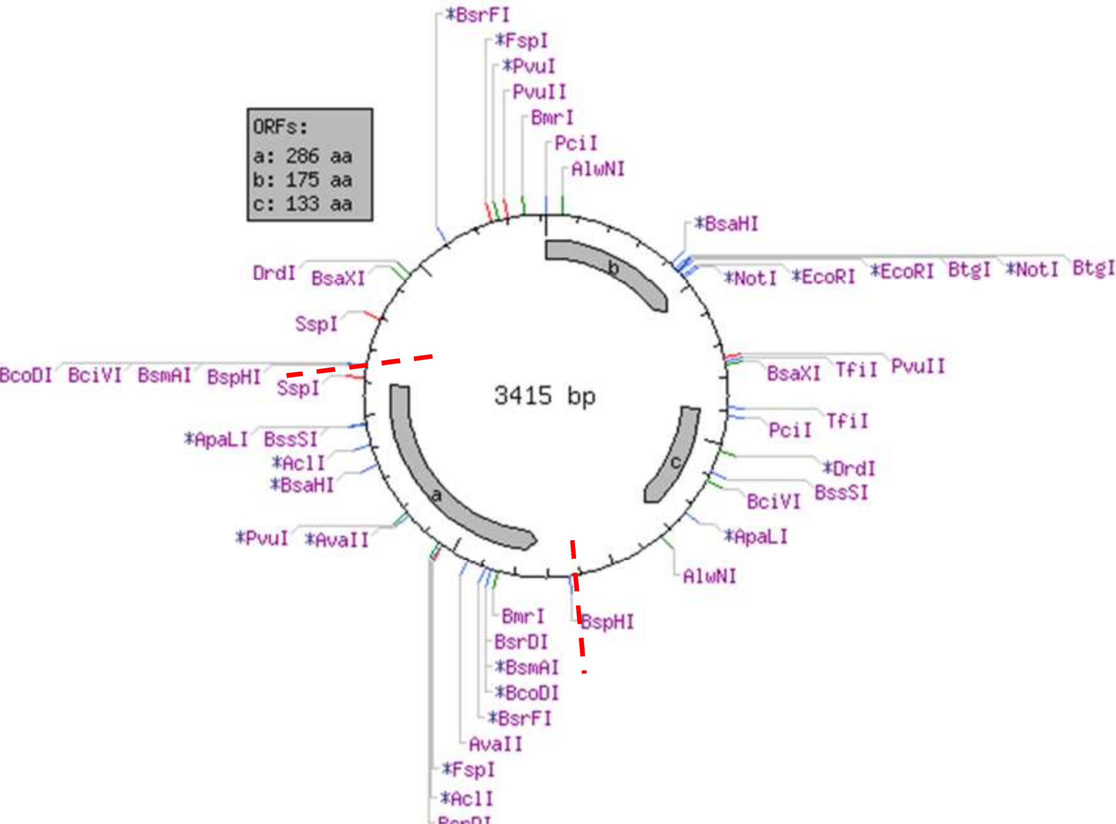


Figure 8.3 NEB cutter visualisation where the enzyme BspHI will cut out Ampr resistance gene for cauliflower 1 and 3. Red line indicates target for enzyme.

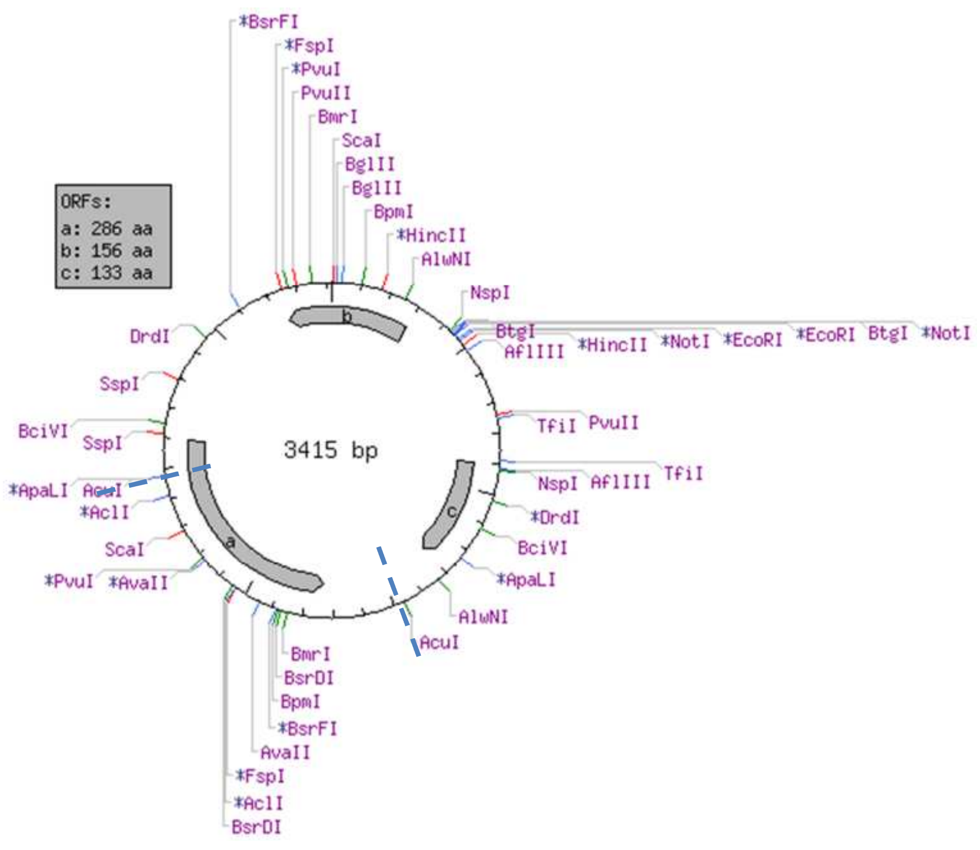


Figure 8.4 NEB cutter visualisation where the enzyme AcuI will cut out AMPr resistance gene for cauliflower 2. Blue lines indicate target for enzyme.

The enzymes were used to cut out the resistance gene using the restriction endonuclease reactions protocol provided by New England Biolabs, and digestion was allowed to occur for 15 minutes at 37°C for both enzymes. The reactions were terminated by heat inactivation. Once this step had been completed, the plasmid DNA was frozen at -70°C to prevent any degradation until further use.

Following digestion with the enzyme, the entire product was loaded into a large agarose gel, with 20ul product into each well. Gel electrophoresis was run as per section 8.3.1.6. Target bands could be isolated under UV light on the gel. The resistance gene was separated into a smaller fragment, and the target fragment was selected and cut from the agarose using a sterile scalpel, aseptically transferred into

1.5ml tubes. The gel plugs were frozen until further processing. Extractions from the gel were undertaken using the QIAquick gel extraction kit from QIAGEN (UK) following the manufacturer instruction.

8.3.1.6 **PCR METHOD**

Biomix Red (Bioline, UK) and the separate Qiagen PCR kit (QIAGEN, UK) were tested for PCR. The clearest bands were created using the Biomix Red. Mastermix was created as per protocol from Bioline. Samples were then run in the Prime thermocycler (Techne, UK). Mastermix and primers were handled only within a PCR workstation (Analytik Jena, UK).

Reactions were as follows;

Stage 1 (initial denaturation) – 1 cycle – 95°C for 3 minutes

Stage 2 40 cycles – (denaturation) 95°C for 15 seconds, (annealing) 60°C for 30 seconds, (extension) 72°C for 30 seconds

Stage 3 (final extension) – 1 cycle – 72°C for 7 minutes

Hold – 4°C

Samples were held at 4°C until running on a 1.5% agarose gel created using molecular grade agarose (Bioline, UK) and Tris-borate-EDTA (TBE) buffer (Thermoscientific, UK) diluted to 1x concentration. Gel was suspended in 1x concentrations TBE buffer and samples were loaded. Ladder was loaded, created using Quick-Load 100bp DNA Ladder (New England Biolabs, UK) and Gel Loading Dye 6x Purple (New England Biolabs, UK). Trouble with ladder smearing on the gel was resolved by altering the concentration of the ladder dye, and diluting with water prior to use. Following loading, the gel was run under electrophoresis (Biometra, Analytik

Jena, UK) at 120 volts for approximately an hour or until dye had travelled two-thirds length of the gel. Bands were visualised by removing the gel from the buffer and placing under UV light at 302nm (Alphamager Mini, Proteinsimple, California).

For qPCR, samples were processed with the setting for Syber Green reactions on the 7500 qPCR (Applied Biosystems, UK) or the micPCR system (Biomolecular systems, Australia). The Lunar Universal (New England Biolabs, UK) Master mix was used for all runs. Reaction volumes were as follows:

Table 8.4 qPCR reaction volumes for each well for hospital samples or all other samples

Reagents	**Reaction (µl)**	**Reaction (µl)**	**Final concentration**
	Hospital samples only	**All other samples**	
Sample	9	1	-
Forward primer	0.5	0.5	0.25 µM
Reverse primer	0.5	0.5	0.25 µM
Luna® Universal master mix	10	10	1x
Molecular grade water	-	8	-
final volume of:	20	20	-

Runs were as follows;

Primer pair 1 and 2

Stage 1 (initial denaturation) – 1 cycle - 95°C for 1 minute

Stage 2 40 cycles – (denaturation) 95°C for 15 seconds, (annealing) 60°C for 30 seconds

Melt curve stage - 95°C for 15 seconds, 60°C for 1 minute, 95°C 30 seconds, 60°C for 15 seconds

Primer pair 3

Stage 1 (initial denaturation) – 1 cycle - 95°C for 1 minute

Stage 2 – 40 cycles – (denaturation) 95°C for 15 seconds, (annealing) 64°C for 30 seconds

Melt curve stage - 95°C for 15 seconds, 60°C for 1 minute, 95°C 30 seconds, 60°C for 15 seconds

8.3.2 OLIGONUCLEOTIDE VALIDATION METHODS

8.3.2.1 VIABILITY OVER TIME

The longevity of the oligonucleotide marker must be determined in order to assess the period in which it could be traced across clinical surfaces, as well as confirm the stability over time. To assess this, ceramic tile surfaces were used as a representative non-porous surface to replicate similar surfaces found within the clinical environment. Ceramic tiles were prepared as in section 3.3.2. 100µl at 9,121copies/µl of one of the oligonucleotide markers, as a representative, was inoculated onto a surface and allowed to fully dry. During the test, temperature and humidity were recorded, but not controlled (appendix). Quadruplicate samples were taken with the swab method as per section 3.3.4.2 at the following time intervals; 0, 2, 4, 6, 8, 24, 48 hours, and 7, 14, and 30 days. Swab tips were placed into 1ml molecular water and 1ul was taken for processing using qPCR (section 8.3.1.5).

8.3.2.2 DNA TRANSFER AND HAND HYGIENE

In order to assess how the oligonucleotide would be transferred across clinical spaces it was important to determine how hands would transfer the oligonucleotide across surfaces, and how many subsequent surfaces it would be detectable from once, being picked up from a healthcare workers' hand, both with or without gloves.

Ceramic tiles were prepared as per section 3.3.2. These were used as representative fomites for transfer. 100µl of oligonucleotide marker at 9,121copies/µl was inoculated onto a surface and allowed to fully dry. During the test, temperature and humidity were recorded, but not controlled (appendix). This was then touched with a hand, gloved or bare skin, using firm pressure on the fingertips, and pressed against 10 surfaces in succession. Swabs were taken as per the same method for bacterial sampling, in section 3.3.4.2, from the whole surface of the hand, and processed by qPCR as method in section 8.3.1.6. Transfer of copies could be calculated.

Further to transfer, removal from hands must be considered in the form of hand hygiene. This was addressed by assessing how the oligonucleotide was removed from artificially contaminated hands, both with and without gloves, by using hand hygiene adhering to the 5 moments or alcohol hand gel.

Bare hands or gloved hands were inoculated with 100µl (3.98E+08 copies/µl) oligonucleotide in 10x10µl drops and allowed to dry fully before challenge with hand washing or alcohol hand gel. Hand washing followed the NHS guidance for washing hands, which requires 11 steps to achieve safe hands (APPENDIX). Hand gel was applied using the same guidance, which requires 8 steps to achieve safe hands (APPENDIX). A systematic review of the literature reported that compliance to hand hygiene is usually around <60% [423]. Following hand hygiene, a swab of the entire hand surface was taken and processed immediately with qPCR method section 8.3.1.6.

8.3.2.3 **DNA WIPE EXPERIMENT**

In addition to removal by hand transfer and hand hygiene, removal of the oligonucleotide will occur by surface cleaning. Furthermore, it is important to assess how the oligonucleotide is cleaned in comparison to pathogens of clinical concern. To be an effective model for infection transmission, the oligonucleotide should be removed in a similar fashion to organisms, or its limitations clearly identified.

To assess this, ceramic tiles were used to represent non-porous surfaces. They were prepared as in section 3.3.2 and inoculated with CaMV as per section 4.3.2, using CaMV as a surrogate for bacterial organisms, and allowed to dry. Tiles were wiped with either a single wipe in one, fluid motion with firm pressure, or two consecutive wipes. The following wipes used to clean NHS surfaces were assessed: green Clinell universal wipes, alcohol wipes, Clorox wipes, and a gauze control wipe was also used to assess physical removal in the absence of an active ingredient. A contact time of 10 minutes was allowed for all cleaning agents. Following contact time, swab samples were taken and processed by qPCR as per section 8.3.1.5.

8.4 Results

qPCR assay efficiencies results are provided in APPENDIX

8.4.1 Validation results

8.4.1.1 Viability over time

The results showed that the oligonucleotide marker, when tested under laboratory conditions recovered from a ceramic tile surface, could remain detectable for up to 7 days, with no detection at 14 or 30 days. This provides a maximum timespan for the experiment design, as well as the confirmation that the marker will not persist in the clinical environment following the completion of the study.

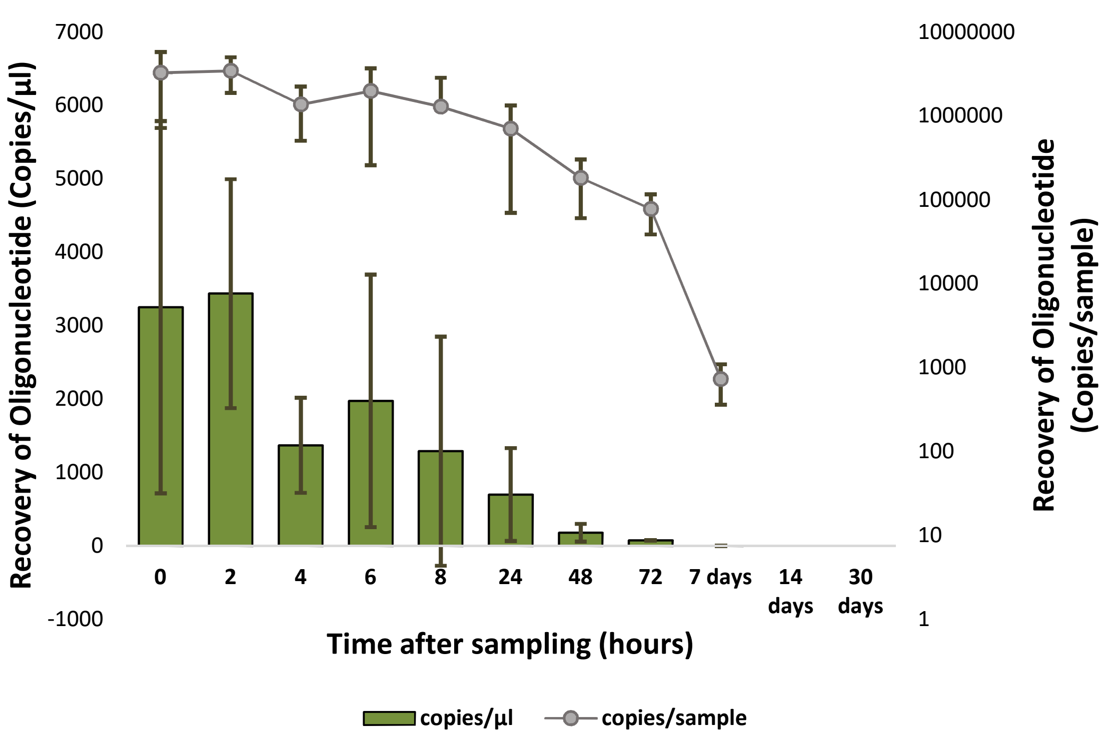


Figure 8.5 Average (mean) Recovery of oligonucleotide over time from ceramic surfaces

8.4.1.2 **DNA CLEANING**

The results showed one instance of hand hygiene, either hand washing adhering to the 5 moments for hand hygiene or using hand gel with >60% alcohol content rendered the oligonucleotide undetectable (appendix). This is comparable to pathogens found in the clinical environment, which would be destroyed or rendered inactive by proper hand hygiene. For removal from a surface, it was found that the DNA marker could be removed with cleaning, and log reduction varied depending on type of wipe used and if one or two wipes were used (1.95-2.80). These results can be compared with the data retrieved for the same testing using 4 clinically significant pathogens in chapter 4.

Table 8.5 Removal of oligonucleotide marker from tiled surfaces following cleaning with different wipes with a single, or multiple wipe

Wipe type	Control Copies/µl	Test Copies/µl	log reduction	% reduction
Suspension (no wipe control)	9.12E+03			
Clinell 1 wipe	9.12E+03	102.55	1.95	98.88
Clinell 2 wipes	9.12E+03	33.72	2.43	99.63
Clorox 1 wipes	9.12E+03	17.28	2.72	99.81
Clorox 2 wipes	9.12E+03	17.07	2.73	99.81
Alcohol 1 wipes	9.12E+03	24.19	2.58	99.73
Alcohol 2 wipes	9.12E+03	14.60	2.80	99.84
Gauze 1 wipe	9.12E+03	48.44	2.27	99.47
Gauze 2 wipes	9.12E+03	36.45	2.40	99.60
2 hour drying (no wipe control)	9.12E+03	1124.98	0.91	87.67

8.4.1.3 DNA TRANSFER

Under the conditions applied within this test, it was found that gloved hands transferred more of the DNA marker than bare hands. On average (mean), gloved hands transferred 45,000 copies to a ceramic fomite, and hands without gloves transferred between 21,810-20,460 copies. The oligonucleotide marker could be transferred to 6 surfaces following a single touch from a contaminated surface. These findings show that a single touch without an instance of hand hygiene could inoculate up to 6 surfaces with the surrogate.

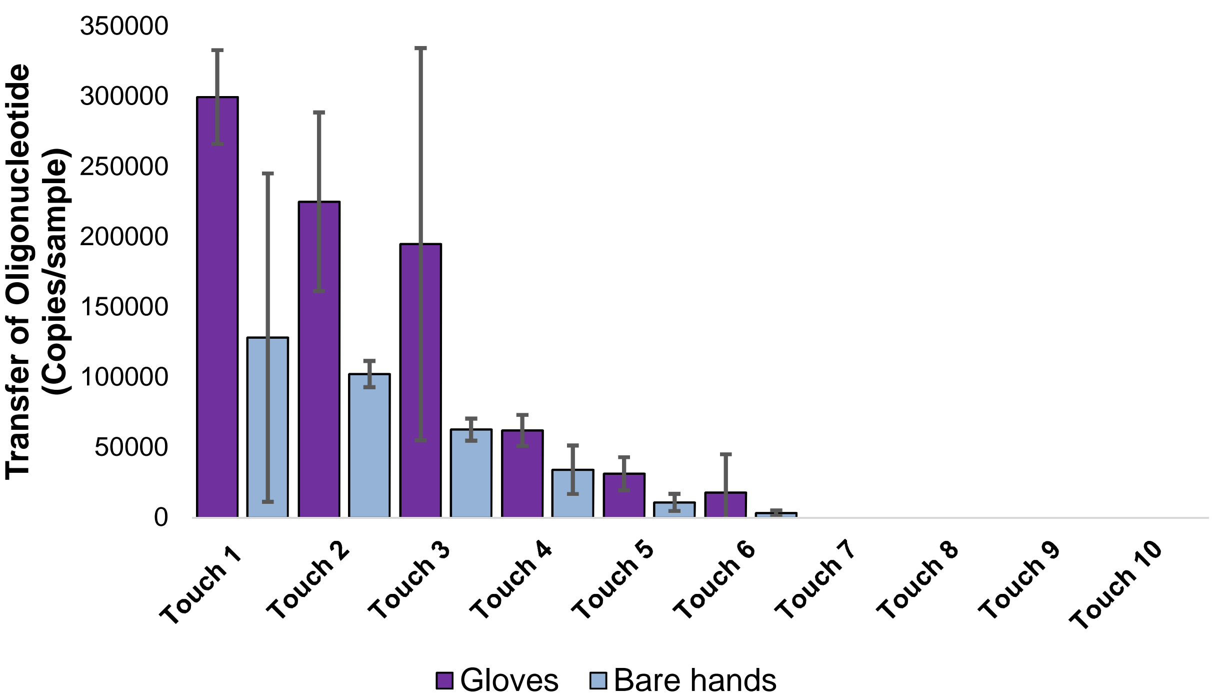


Figure 8.6 Transfer of oligonucleotide following touch from a contaminated surface onto 10 consecutive surfaces with gloved or non-gloved hands (copies per swab sample).

8.5 DISCUSSION

Surrogates for infection are commonly used in experimental design. When use of the original infectious agent would prove unsafe or difficult, the nearest surrogate can be used. This is common when testing disinfectants, such as the use of feline calcivirus as a surrogate for norovirus [424], or animal viruses in place of human counterparts. Both cauliflower mosaic virus DNA and bacteriophage MS2 have been proven as safe and effective markers for dissemination studies within hospital environments. While both proven effective [425], CaMV markers have the advantage of lasting longer on surfaces and the possibility of using multiple markers with different primer pairs simultaneously [425], as was undertaken in this chapter.

While safe and effective, it is important to understand the associated limitations with the CaMV marker. Recovery of the surrogate DNA cannot imply that infection would occur. Recovery by qPCR cannot determine viability and will not differentiate between live or dead cells, or viable and non-viable DNA. Furthermore, the amount of marker recovered does not necessarily mean that quantity could be sufficient to cause clinical infection if it were an infectious material. Infectious dose for different organisms varies greatly, and sometimes, a larger amount of contamination would be required to cause clinical infection. Noroviruses have very low infectious doses, at ≥18 viral particles [426]. Similarly, *C. difficile* has been shown to cause infection with just 1 CFU in mouse models [90]. In contrast, *S. aureus* requires a minimum of 100,000 CFUs [427]. However, the recovery of the marker does indicate a failure in cleaning and hand hygiene, and highlights how just a single inoculation of a marker on one surface can spread rapidly [186].

When assessing the transfer of the surrogate with bare hands or gloved hands, it was found that gloved hands transferred more of the marker. Poorly fitting gloves can provide a greater surface area for potential organism adherence. Surface-to-fomite and fomite-to-surface transference interactions are complex, and vary depending on surface type, pathogen type, glove material or skin condition. Estimating possible transfer can be difficult, though some comparisons have been made for *A. baumannii* in which reduction in bacterial transfer occurred with gloves compared to bare skin [428], and findings where fewer bacteria adhered to bare hands when compared to gloves, tested against a mixed bacterial culture sourced from a beef carcass [429]. In a study using circular CaMV, with bare fingertips, transfer was seen after 9 touches onto stainless steel discs [425]. For linear CaMV, tested on ceramic surfaces for this study, it could be recovered after 6 touches.

8.6 CONCLUSION

This study showed how the CaMV-based DNA marker proved an effective and useful tool for tracking how an infectious agent might move across hospital surfaces, and to determine how effective current cleaning efforts were. The marker can be removed using routine surface cleaning products, and was also undetectable following an instance of proper hand hygiene, either washing with soap and water or using an alcohol-based hand gel, adhering to the 5-moments for hand hygiene. The marker can be produced in larger quantities, inoculated onto surfaces and easily recovered with swabs, and quantified with PCR.

Chapter 9 TRACKING INFECTION MOVEMENT WITHIN A HOSPITAL WARD

9.1 INTRODUCTION

Hospital ward environments are busy and dynamic. Patient turnover, visitors and staff movements all facilitate the movement of infection within these environments. The hospital environment is known to play a role in the transmission of pathogens, and as the environment is consistently exposed to pathogens shed from patients and other ward users, it can be difficult to eliminate this risk, and organisms can persist on surfaces and in the air [430].

Ward environments are designed to prevent this transmission, and the infrastructure of the hospital has an impact on infection prevention and control (IPC) [431]. Such examples of design including the availability, placement and accessibility of alcohol gel dispensers, which play a role in how often they are used. Appropriate placement, adjacent to the patient in clear view, saw use at 53.8%, opposed to just 11.5% use when the dispenser was installed at the door [432]. Availability of single-patient rooms reduces colonisation risk, and increased distance between patient beds reduces droplet transmission risk and allows movement of healthcare workers and cleaners [431]. Sinks are critical for hand hygiene. Easy access and visible placement [433] as well as appropriate use and design to prevention contamination of hands or surrounding areas is important [434].

As there are many factors and building design elements that can change how an organism may move around the clinical environment, it is important to know how, for each specific ward environment, a pathogen may move. Sink surfaces are an

important component of safe hospital design, and poor sink placement have IPC implications. Sinks are known reservoirs for clinically significant pathogens, particularly those that thrive in damp environments, such as K. pneumoniae and P. aeruginosa, which have been isolated from sinks. Poor sink design or placement can aid transmission of these organisms. While it is important to know sinks pose a risk, further insight is needed into the true problem. Knowing how transmission occurs from the sink to the wider environment, outside of the direct use of the sink, should be considered. A study by Buchan *et al.* 2019 found that following a culture assessment of a 600-bed hospital, a high prevalence of carbapenemase-producing *K. pneumoniae [29]*. Studies sampling sinks and drains for *K. pneumoniae* are not novel, though this study did identify a design flaw. Sinks near toilets were 4-times more likely to be positive (87% of samples) than sinks distal to toilets (22%) [29]. This highlights that while cleaning is the key to preventing such surfaces acting as reservoirs, the design of the surface environment plays a role too. The problem with HCAI is multifaceted, and transmission routes should be carefully identified to further highlight such issues outside of cleaning failure, such as design flaw. While it is easy to blame poor cleaning, there are undoubtedly other factors at work during transmission events. Identifying and being aware of these specific routes of transmission can identify sentinel sites for surface sampling, either when routine monitoring of the environment or provide a first point of sampling in an outbreak scenario, as well as help risk assessment for cleaning different surfaces. Knowing how organisms move and are deposited can identify where heightened cleaning efforts should be, and identify surfaces that have higher levels of contamination, such as the

bookcase in a ward environment (chapter 6). Additionally, tracking this movement can identify potential instances where hand hygiene has failed, either by compliance issues or ward design flaw, such as poor placement of alcohol gel dispensers.

9.2 RESEARCH AIMS

The oligonucleotide was released in a ward environment and tracked, to assess movement and cleaning efficacy. The following aims complete the work within this chapter:

1. Release an oligonucleotide marker within a ward and identify how this marker has been moved within a clinical space.
2. Use these findings to advocate for enhanced environmental monitoring and cleaning.

A component of these results were used and presented as a letter in the Journal of Hospital Infection, in light of the SARS-CoV-2 outbreak, to help emphasise the importance of surfaces in relation to infection control and transmission within clinical spaces [186].

9.3 METHODS

9.3.1 INOCULATION OF THE SURROGATE MARKER

The three oligonucleotides were released within a ward, with samples taken every day for 5 days. Three surfaces were chosen and a different oligonucleotide marker inoculated on each;

- marker 1 on a bed rail in isolation room,
- marker 2 computer mouse in a treatment room,
- and marker 3 on the children's play table in reception.

Inoculations consisted of 100µl of each oligonucleotide marker at 1.15E+09 copies of linear plasmid DNA diluted in molecular grade water. Surfaces were inoculated at 7:30am on Monday morning and allowed to move for a full day cycle (10 hours) within the ward before samples were taken to recover the oligo at 5pm to assess movement and cleaning. The samples were taken prior to the cleaning, which was undertaken with 1,000ppm chlorine, which has been demonstrated to degrade DNA.

9.3.2 SAMPLE RECOVERY AND PROCESSING

Surfaces were sampled as previously described in section 8.3.1.6 and assessed with qPCR. The following surfaces formed the daily sampling plan;

Table 9.1 Daily sampling plan for ward surface sampling

Sample number	Surface	Area
1	Bed 1 rail right	Bay 1
2	Bed 2 rail right	Bay 1
3	Bed 3 rail right	Bay 1
4	Bed 4 rail right	Bay 1
5	Bed 5 rail right	Bay 2
6	Bed 6 rail right	Bay 2
7	Bed 7 rail right	Bay 2
8	Bed 8 rail right	Bay 2
9	Bed 9 rail right	Bay 3
10	Bed 10 rail right	Bay 3
11	Bed 11 rail right	Bay 3
12	Bed 12 rail right	Bay 3
13	Bed rail right	Cubicle 1
14	Door handle exit	Cubicle 1
15	Door handle toilet	Cubicle 1
16	Over bed table	Cubicle 1
17	Bed rail right	Cubicle 2

18	Door handle exit	Cubicle 2
19	Over bed table	Cubicle 2
20	Door handle toilet	Cubicle 2
21	Door handle exit	Cubicle 3
22	Bed rail right	Cubicle 3
23	Over bed table	Cubicle 3
24	Toilet door handle	Cubicle 3
25	Bed rail right	Isolation room
26	Door handle exit	Isolation room
27	Bed table	Isolation room
28	Toilet door handle	Isolation room
29	Kids table	Reception seating
30	Book	Playroom
31	Dollhouse	Playroom
32	Shelf	Playroom
33	Prep surface	Treatment room 1
34	Trolley handle	Treatment room 1
35	Prep surface	Treatment room 2
36	Chair arm	Corridor
37	Trolley handle	Treatment room 2
38	Bookcase	Corridor
39	Keyboard	Height and weight room
40	Nappy change	Height and weight room

41	Door handle exit	Height and weight room
42	Notes trolley	Main reception
43	Keyboard	Main reception
44	Chair arm	Reception seating

These surfaces were sampled where possible, though in a dynamic ward environment, this was not always possible. If some surfaces were unavailable for any reason, the nearest alternatives were sampled instead. Any deviations from the original sampling sites are listed in appendix. Samples were taken in a 10x10cm^2 area, as per section 3.3.4.2. For smaller surfaces such as door handles, the entire surface was sampled. Samples were stored and transported in an insulated cool box on ice, kept refrigerated (2-10°C) until processing. All samples were processed within 3 hours by qPCR, as in section 8.3.1.6.

9.4 RESULTS

The transmission of the DNA marker within the ward were animated to visually explore how the surrogates moved across the different surfaces:

Marker 1: https://youtu.be/haVPliuQ4WU

Marker 2: https://youtu.be/yxMvGAoB7XQ

Marker 3: https://youtu.be/DuIh932T3Gg

Following the release in the ward, the three surrogates spread across the surfaces and were located on different surfaces across the ward environment.

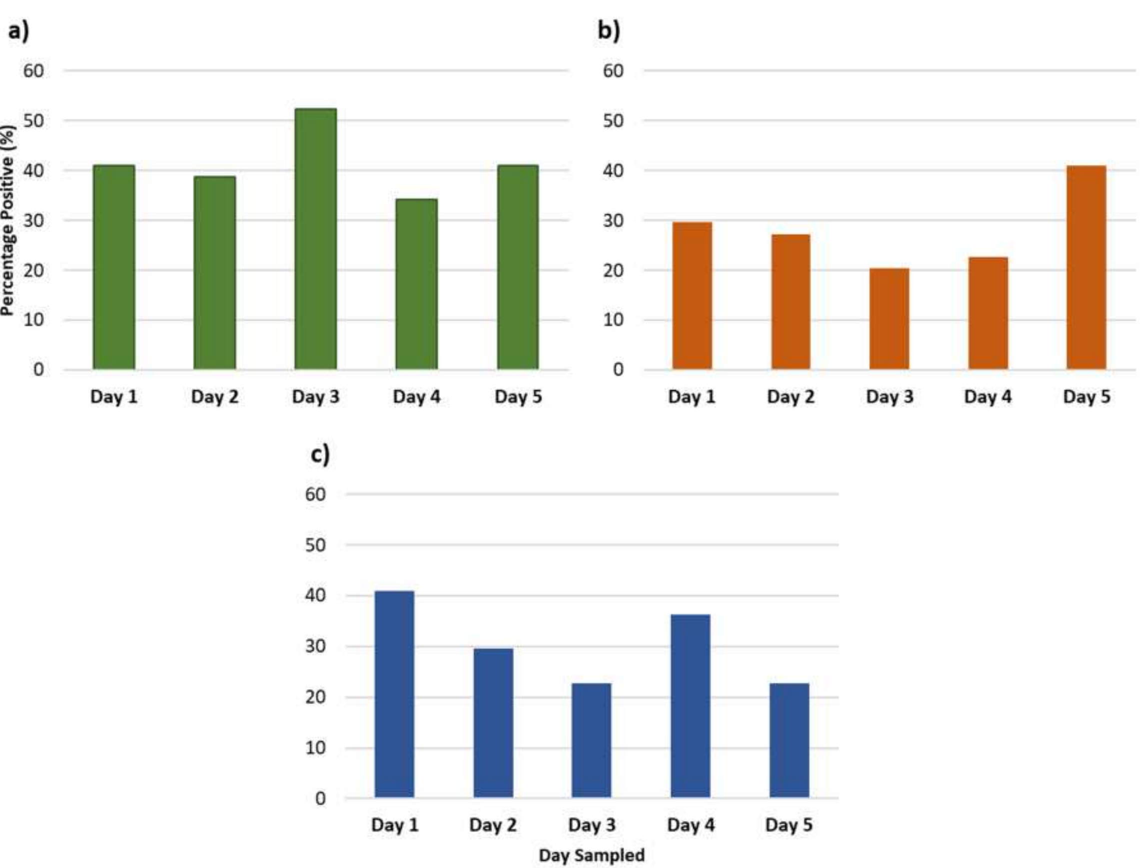


Figure 9.1 Average (mean) percentage positive sites for oligonucleotide 1 bed rail in isolation room (a), 2 computer mouse in treatment room (b) and 3 play table in reception (c) across the 5 day sampling period.

The results showed that the surrogate markers spread quickly through the ward environment. Within 10 hours, on average (mean), 41%, 30% and 41% of surfaces had some contamination with markers 1, 2, or 3 respectively. At the final sampling point, day 5, 41%, 41% and 23% of sites were positive with markers 1, 2 and 3. Across the entire sampling effort, only 3 surfaces (a bed table in cubicle 1 and a bed table and a door handle in the isolation room remained uncontaminated with any marker throughout the 5 day sampling process.

The ward area can be broken down into different areas; the immediate bedspace, the wider bedspace, clinical areas, and general ward areas. When exploring percentage of sites showing contamination with the marker, it can be seen that the type of surface plays a role in how many sites are contaminated.

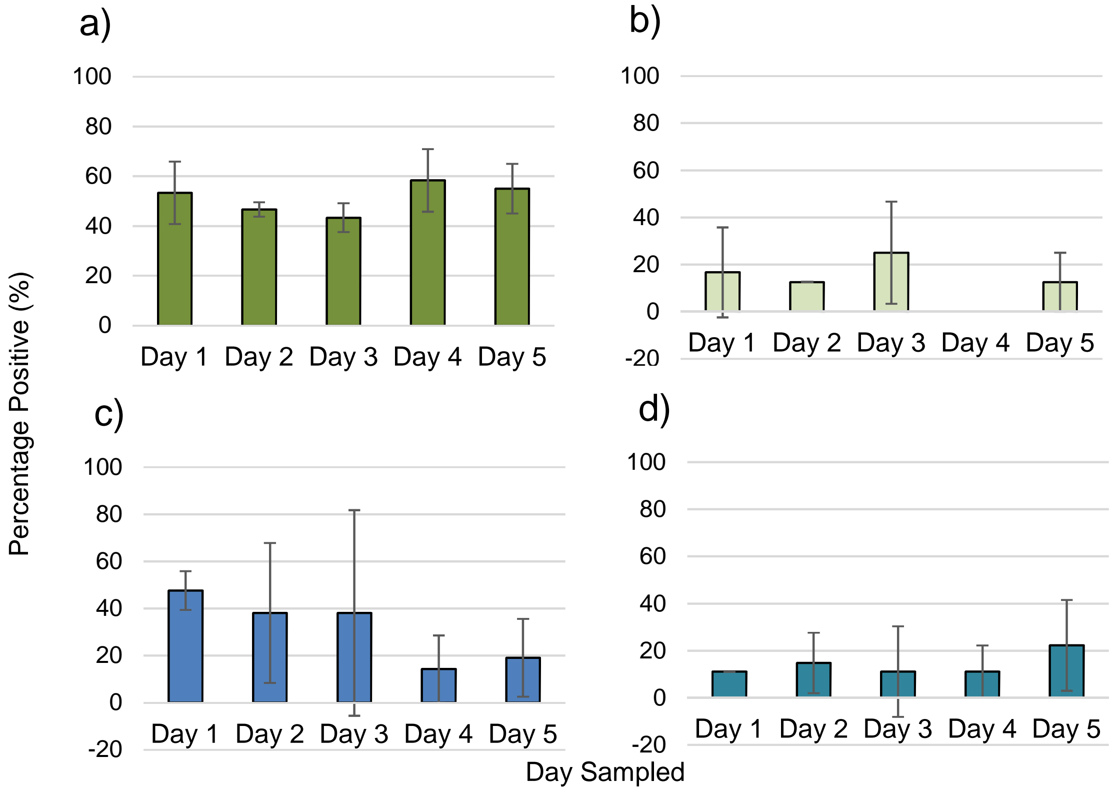


Figure 9.2 Percentage positive sites for all three surrogate markers, recovered from a) immediate bedspace environment b) wider bedspace c) clinical areas d) general ward surfaces.

Some areas had more positive sites than others. The general ward areas had the fewest positive sites. The most positive sites were found within the immediate bedspace environment. Surfaces within the immediate bedspace had the most positive sites overall, with 53.33% surfaces showing marker contamination on day 1, and 55% at day 5.

For wider bedspace surfaces, fewer positive sites were found. On day 1, just 16.66% of sites were positive. On day 4, no sites were found positive, and day 5 had just 12.5% sites showing contamination. Clinical areas had more positive sites than wider bedspace areas or general ward areas, though not as many as the immediate bedspace areas. On day 1, 47.62% of surfaces were positive, falling each day until day 4, in which 14.29% sites were positive, with a small increase in day 5 at 19.05%. General ward areas, like wider bedspace surface, had fewer contaminated sites. On day 1, only 11.11% sites were positive, reaching a maximum of 22.22% on day 5.

Cleaning responsibilities are divided into different staff groups, and different surfaces require different levels of clean. General areas, such as floors and walls within the wider ward and reception areas are not classified as high risk. The cubicles require deep cleaning by domestic workers, the bays had general daily cleaning by domestic workers and healthcare assistants, and the other non-clinical surfaces had light cleaning where required to uphold the visibly clean standard.

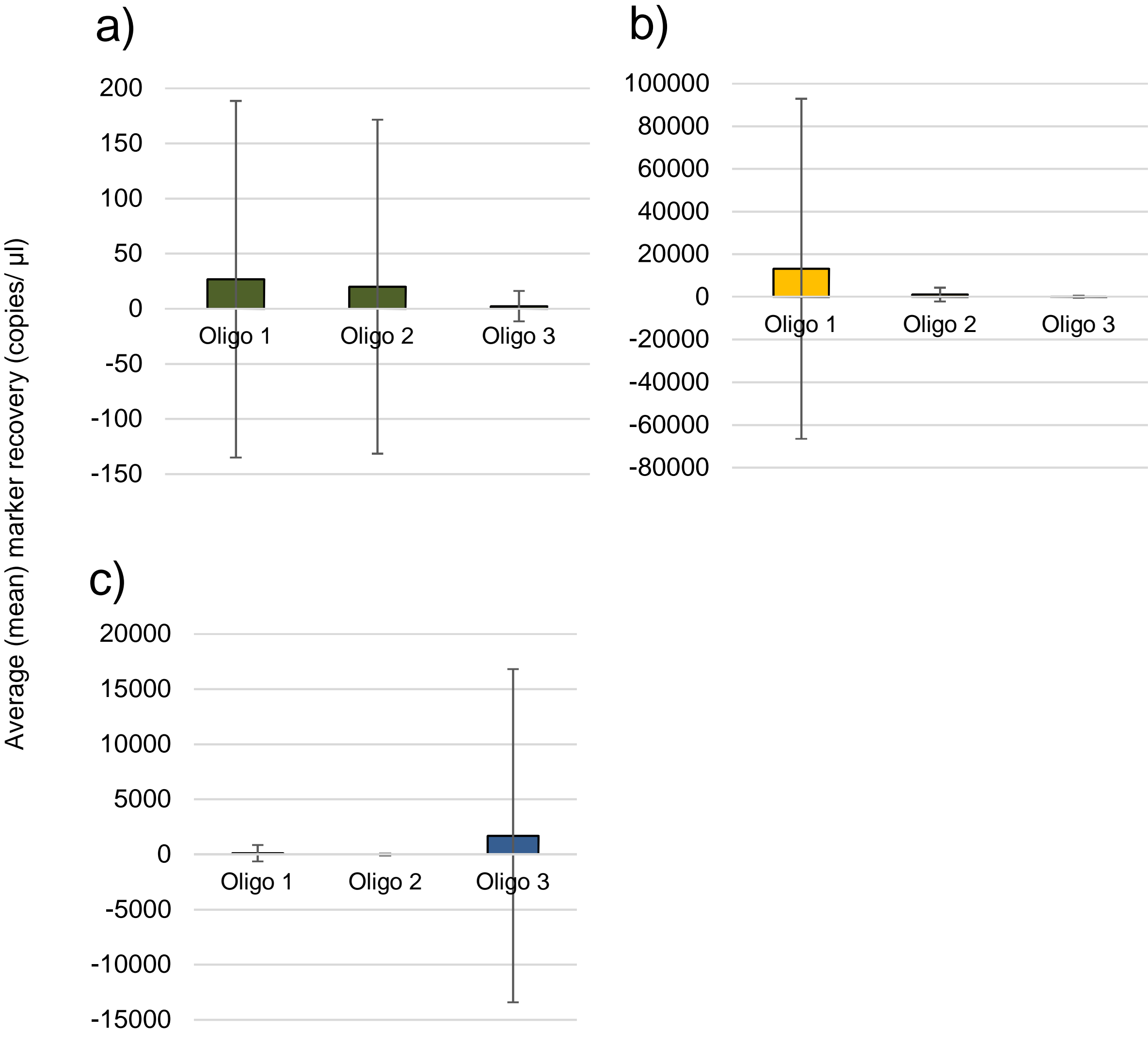


Figure 9.3 Average (mean) recovery of oligonucleotide marker across all sampling days from surfaces within a) cubicles b) bed bays c) all others

When exploring the difference between DNA marker recovery and cleaning type within each area, it was found that the cubicles had the lowest marker recovery, at an average (mean) 16% positive, as well as the lowest concentrations of marker, with an average of 26.72, 20.03 and 2.32 copies/µl for markers 1, 2 and 3 respectively. The bays were associated with highest percentage recovery, at 72% of sampled surfaces showing contamination, and an average recovery of 13,175.88, 1095.79, and 127.73 copies/µl for markers 1, 2 and 3. All other surfaces had a low

positive rate, at 22% positive, and a moderate level of recovery of the marker, with 114.50, 17.16, and 1702.96 copies/µl for markers 1, 2, and 3.

9.5 DISCUSSION

This study sought to address how an infectious material might move around the clinical surface environment, by use of a surrogate marker. By using a DNA surrogate based on the cauliflower mosaic virus, movement and transfer of the surrogate could be quantified and qualified by sampling surfaces with swabs and analysing the samples with PCR. Three distinct sections of CaMV with specific primer pairs allowed the inoculation of different sites within the ward with the distinct markers, that could be defined during the analysis, to see how the surrogate moves from the three sites and the interactions between the different surfaces.

Within 24 hours, 41%, 30% and 41% of surfaces were contaminated with markers 1, 2 or 3. Each of the three markers travelled throughout the ward. Surfaces were divided into near patient (marker 1, bed rail in isolation room), clinical ward (marker 2, computer mouse in treatment room) and non-clinical (marker 3, play table in reception area). Marker 1 travelled, in high concentrations (average 12,162.82 copies/µl across 5 days) to near patients surfaces in nearby bed bays. It was found in non-clinical areas in lower concentrations, such as the playroom surfaces (average 7.05 copies/µl) and reception areas (average 0.88 copies/µl). Marker 2 did not disperse well in the treatment room where it was initially inoculated, with just 2.69copies/µl recovered from the preparation surface on day 1, and no other positive sites for the other 5 sampling days. Marker 2 was recovered in higher concentrations, like marker 1, from the bed rails in the bed bays, at an average 1,095.5copies/µl. Marker 2 did not widely disperse to the general ward or clinical areas, with just 17.16copies/µl average across all sampled sites, with no positive sites recorded for

the reception and reception seating areas, and 69 of the 80 cubicle surfaces sampled. Marker 3 also travelled to each of the 3 bed bays, with an average 127.73 copies/µl across the 5 days. As with marker 2, marker 3 did not disseminate well in the general ward areas, the clinical surfaces or the wider bedspace surfaces, with an average 852.64 copies/µl across all surfaces not within the bed bays.

Recovery of this marker is indicative of either cleaning failure or failure to perform hand hygiene after prior cleaning. This is determined by presence of the marker, as the chlorine is used to clean the ward and chlorine has known efficacy against many pathogens and DNA. Work in chapter 8 also showed how the DNA marker could be rendered undetectable (≥8.13 log_{10} reduction) following use of a bleach-based wipe (section 8.4.1.2).

Some surfaces showed re-inoculation with the marker. Multiple bed rails within the bed bays were contaminated at day 1, had no detection of marker following cleaning and later sampling, but proceeded to present as positive upon sampling later within the week. Some surfaces also remained clean for several days following the release of the markers, but became contaminated near the end of the week, such as the chair arm in the corridor becoming contaminated with markers 1 and 2 at day 5. This also occurred for some surfaces within the cubicles, for marker 2, in which two door handles and a bed table became positive on the final day of sampling only. This re-inoculation of surfaces implies an intermediary surface acted as a reservoir for the marker due to poor cleaning. These missed surfaces, which perhaps not classed as critical themselves, represent the risk that incomplete

cleaning poses to IPC, as interactions in the ward allow the movement of organisms from dirty to clean surfaces.

Different areas and surfaces are allocated to different staff groups to clean. When assessing recoveries, the surfaces requiring the highest level of clean had this enhanced cleaning effort reflected in the results. Cubicles have deeper, more frequent cleans due to their use for isolating shedding patients requiring contact precautions. These surfaces had the lowest % positivity, and far lower concentrations of marker recovery, indicating the frequent and deep cleaning of these surfaces had a positive impact on reducing the frequency of recovery and quantity of marker on the surfaces.

Additionally, some surfaces were found to be contaminated with all three of the markers. All sampled bed rails, aside from a single bed rail in bay 2, were found to be contaminated simultaneously with all 3 of the markers across multiple days. This accumulation of separate markers originating from different surfaces within the ward has implications for not only the quality of the cleaning, but for gene transfer and resistance (see section 1.3.5). Some organisms readily share genes, and the accumulation of different markers, representative of different species, presents the opportunity for pathogens to undergo horizontal gene transfer. With the rise of antibiotic resistance, the surface environment should not to be overlooked, as surfaces can act as a reservoir of resistant genes, increasing the opportunity for clinically significant pathogens to uptake resistance genes [73]. Additionally, many antibiotics persist in the environment, representing an additional risk to sink and sink-adjacent surfaces [435].

9.5.1 MARKER SPREAD AND CLEANING RESPONSIBILITIES

Isolation rooms have a critical role within the hospital setting. These rooms are used to house immunocompromised patients to keep them safe from outside sources of infection, or to isolate an infectious shedding patient. These rooms have specific design features to ensure a patient is protected [436]. Positive pressure reduces the risk of outside contaminants entering the room and reaching the patient, whereas negative pressure prevents inside contamination from entering the wider ward. Isolation rooms have a smooth, seamless and minimalistic design to allow frequent and easy cleaning, excellent sealing between the walls and floor [436], and usually have filtration systems air is passed through before being vented outside. Isolation rooms come complete with an ensuite bathroom so the patient can remain inside throughout their stay. With the current pandemic and the concern of limiting the spread of COVID-19 within hospitals, the importance of isolation rooms and keeping them clean, and how to clean after an aerosol generating procedure, is of concern [437, 438]. A systematic review of the literature by Barlow *et al.* 2006 found that isolation policies in combination with other infection control measures can reduce MRSA transmission [439]. In addition to this, a study of a 215-bed hospital found that enhanced cleaning of high touch surfaces within isolation rooms of *C. difficile* infected and MRSA colonised patients led to reduced acquisition of these pathogens on healthcare workers' hands caring for these patients [440]. As previously discussed throughout this thesis, cleaning is one of the most critical IPC measures to prevent the spread of hospital infection. However, without good hand hygiene, clean surfaces will become re-contaminated and critical areas, such as isolation rooms, will not work as they should. Isolation rooms are often linked to

colonisation of patients. Colonised patients show increased environmental contamination than infected (0-106 CFU/cm^2 against 0-29 CFU/cm^2 respectively) [93]. As isolation rooms are used to house infectious and colonised patients, cleaning is critical to ensure the safety of both the clinical staff and visitors entering the isolation room, the wider ward users, as well as the next patient to be housed within that same isolation room following a discharge. If hand hygiene is not being undertaken, then isolation rooms cannot perform their function. Within the context of the paediatric ward sampled within this study, a hematology-oncology ward, spread of organisms, or a surrogate, has implications for patient safety within the rest of the ward, as the patient subset is immunocompromised. As these are such critical rooms and are associated with a higher risk of surface organisms and multi-drug resistant organisms (MDRO), the cleaning procedure for these rooms is more frequent and comprehensive than the other spaces within the ward. As such, this was selected as one of the inoculation sites for this study.

The surrogate inoculated onto the bed rail within the isolation room should, if cleaning protocol and hand hygiene is impeccable, should not leave the isolation room. Frequent cleaning should remove or dramatically reduce the amount of marker within the room. Movement of the marker from the bed rail to the outer areas of the ward demonstrates a lack of effective cleaning and hand hygiene, as the cleaning agent used within the ward is known to be effective against DNA [441], and the marker was shown to be removed by common cleaning agents (section 8.4.1.2) or an instance of hand hygiene (section 8.3.2.2). The marker moved, within 1 day, outside of the isolation room and into the neighboring bed bays. The highest

concentrations of marker were found in the nearest bays (1 and 2) and the concentration reduced in bay 3. The marker was found in low concentrations is most other areas of the ward. This demonstrates poor hand hygiene as clinical staff move out of the isolation room to attend to other patients within the bays. As the marker was found in the bays for the 5-day sampling period, this issue has been worsened by poor cleaning or the misunderstanding of cleaning roles and what has or has not been cleaned by other team members, as also found during a cleaning observation study (chapter 7). If this marker were an infectious disease, such as SARS-CoV-2, this could have drastic implications for clinical infection, though no comment can be made on the amount of marker retrieved and how this may translate to an infectious dose.

9.5.2 WHO IS FACILITATING THIS MOVEMENT?

The original inoculation sites were carefully chosen to represent different movements within the ward, and show the difference between clinical and non-clinical movements. Inoculation site 1, the bed rail within the isolation room was chosen to show how people entering and leaving the isolation room, mostly staff, would transfer the marker. Marker 2, inoculated on the mouse in reception will be used by staff only. The marker placed on the children's play table in the reception area is likely to be touched only by the children (patients). This is important to see how staff and patients may spread the marker differently, suggesting how one group may be a bigger driver for environmental organism transmission. Staff have consistent and frequent movement between patients, and have frequent contact with potentially contaminated surfaces within the bed space, such as equipment

monitoring equipment, the sink area and the nurses station. These frequent contacts put healthcare workers at higher probability of facilitating contamination movement through contaminated gloves or hands [442]. As healthcare workers move from patient to patient, good hand hygiene is essential to ensure HCAI is not transmitted. The results show that marker 1, inoculated within the isolation room, readily moved out into the neighbouring bed bays. This movement into the bed bays suggests poor hand hygiene as the marker is transmitted between patients. The marker is also found in much lower concentrations in the wider ward environment, such as in the reception. The marker remained detectable within the bed bays for all 5 sampling days, suggesting cleaning with not effective, and other surfaces may be acting as an intermediary reservoir as the marker continues to be deposited within the bed bays.

9.5.3 IMPLICATIONS FOR PATIENTS

The study setting was a paediatric haematology-oncology ward. This represents a particularly vulnerable patient subset. Patients visiting the ward and receiving treatment, particularly those receiving chemotherapeutic agents, will be immunocompromised and at higher risk for HCAI acquisition. Additionally, paediatric patients interact differently with their environment than adult patients. Environmental infection control is always critical, however some surfaces are generally overlooked as less important, such as walls, floors, and surfaces lower to the ground. Paediatric patients have greater and more unusual and less restrained interactions with the surface environment and toys and objects within playrooms and reception areas [384, 443], therefore finding the marker present on these

surfaces has greater implications for patient safety within this setting than perhaps an adult ward.

The marker moved quickly out of the isolation room into the bed bays, and was recovered from almost all the bed rails within each of the three bays. Bed rails are often a focus during environmental sampling studies. They are classified as a high touch surface, are near the patient, and represent risk as they are frequently touched by both patient and healthcare workers. An observation study by Cheng *et al.* 2015 recorded the most touches per hour (mean 13.6 touches) on bed rails when compared with other high touch surfaces such as bedside tables, bed frames, lockers, and linens. Of all 6,144 contacts recorded throughout the study, 14.6% were touches involving the bed rail [444]. Another study found similar results, with bed rails being the most frequently touched high-touch surface (mean 7.76 contacts per interaction) when compared with other surfaces such as the bed surface or the bed side table [445]. Comparing this with other studies sampling hospital surfaces where bed rails are repeatedly reported as highly contaminated [122, 123, 126] these findings show that movement involving bed rails have implications for patient safety, as they are interacted with so frequently by both patients and healthcare workers and may lead to transmission events and possible clinical infection, worsening the rate of HCAI and requiring additional interventions and bed days. The data suggests hand hygiene was poor for healthcare workers leaving the isolation room. Hand hygiene was also missed when entering the bed bays. As isolation rooms are used to house shedding patients or patients with multi-drug resistant infections, the movement of the surrogate between these two areas could lead to poor clinical outcome if a patient

in a bed bay became exposed to an infectious agent from the isolation room. As a day unit giving outpatient care, there is a high turnover within the bed bays. Multiple patients are seen throughout the day. Movement of the surrogate from the isolation room into these bed bays and the implicated poor hand hygiene could represent a real risk to many of the immunocompromised patients receiving care on this ward due to the high patient turnover.

9.6 CONCLUSION

The results found that the surrogate marker spread rapidly across the ward, and 41, 30 and 41% of surfaces were contaminated with marker 1, 2 or 3 within 1 day. Re-inoculation of some surfaces was seen, suggesting there was either one or more sites acting as a reservoir for infection. Additionally, some surfaces showed recovery of all three of the markers, highlighting the issue of potential gene transfer if the surrogates were indeed pathogenic organisms.

While no estimation can be made for the direct clinical significance of marker recovery such as possible chance of infection, this highlights how cleaning must be improved. Overall, across all the sampling days, just 3 sampling sites had no instance of detectable marker. The re-inoculation, speedy accumulation in the bed bays and high concentrations recovered within near bedspace surfaces shows that cleaning needs to be more effective. There are many issues with cleaning efficacy and user compliance, which have been explored in chapter 6, and these must be considered when developing or implementing training to ensure cleaning is as effective as possible to promote a clean and safe clinical surface environment.

This movement of the marker highlights the synergistic relationships between different pillars of IPC. Isolation rooms are a required intervention for patients that pose a cross-infection risk, functioning to keep the patient safe, or to keep other patients shielded from transmission events. Infected or colonised patient shed more readily into their surface environment leading to higher contamination within such rooms of certain pathogens, such as MRSA or VRE. If cleaning is not being undertaken effectively, then the surface bioburden may be high. For cleaning to be effective, the

cleaning agent or technique used must be optimal. Even if cleaning is being undertaken appropriately, if hand hygiene is not effective, then transmission from outside the isolation room or from within the isolation room to the wider environment, as demonstrated within this study, will reduce the effectiveness of the isolation room itself. Therefore, all components of IPC (contact precautions, environmental monitoring, cleaning, hand hygiene) must be optimised and utilised in order to achieve the best patient outcomes as each factor impacts the other.

Chapter 10 SUMMARY AND CONCLUSIONS

10.1 WHAT DID THIS RESEARCH SEEK TO DO?

This research sought to assess one of the most important tools available to IPC teams to prevent HCAI, cleaning. Wider environmental contamination, detection and control. Effective cleaning is key to keeping surfaces safe for patients. However, knowing how and when to clean, how to train cleaners and how to assess the impact and quality of cleaning and the contamination within the surface environment can be a difficult and confusing task. This research addressed these aims (section 1.2) by exploring how to assess cleaning and the environment with microbiological surface sampling (chapters 2 and 3). Then, work began to address cleaning (chapters 4, 5 and 6) and finally, behavior and training. A summary of the main findings of the research are outlined below.

10.1.1 MICROBIOLOGICAL SURFACE SAMPLING

The review of the literature revealed several issues related to microbiological surface sampling. Most importantly, the clear lack of guidance for sampling of clinical surfaces and setting up an environmental monitoring programme. The lack of evidence-based guidance meant knowing how to effectively monitor the clinical environment can be a difficult and confusing task. A review of the literature sought to pull together the current evidence and efficacies of different surface sampling devices to allow healthcare professionals to make informed choices for their sampling devices.

The literature review revealed the following information for different surface sampling methods:

- Dipslides, a lesser-known surface sampling device performed well and should not be overlooked for clinical surface sampling.
- Sponges work best for recovering *C. difficile*.
- TSA contact plates are effective for recovering *Acinetobacter* and *Pseudomonas* spp.
- Macrofoam swabs performed better for *P. aeruginosa* and *Salmonella abony* than contact plates for recovery from stainless steel.
- Methicillin contact plates were most effective for recovering *S. aureus* from stainless steel, outperforming dipslides and swabs.
- When assessing sampling devices, ceramic surfaces were easiest to recover from, and plastic surfaces proved more challenging.
- Regardless of sampling device, Gram-positive organisms were more readily recovered than Gram-negatives.
- As found within the review, overall, dipslides were the most effective surface sampling device.

This review of the literature highlighted the variability in experimental data for surface sampling devices, and identified the gaps in the literature, leading to the work undertaken in chapter 3, which had the following conclusions:

- The interactions between sampler, surface type and target organism are important, and all must be considered when selecting a sampling device.
- Surface material plays an important role in how well organisms can be recovered from a surface.

- The results suggest, overall, steel allows easiest recoveries of *E. faecalis*, with poor recoveries of *K. pneumoniae* and *P. aeruginosa*. Ceramic surfaces allow good recovery of *K. pneumoniae* and *E. faecalis*. Plastic surfaces allow good recovery of *S. aureus*, but poor recoveries of *E. faecalis* and *P. aeruginosa*. This has implications for the surface material used as a representative (most commonly stainless steel) when testing surface sampling device efficacies, as some species may be underestimated on different materials.
- Organisms have the ability to adhere well to certain surfaces, and their mechanisms of attachment vary. Organism size, shape, charge and ability to form biofilm will all impact how well they are recovered with different sampling devices.

10.1.2 EFFECTIVE CLEANING

Following the work in chapters 2 and 3, recovering organisms from the clinical environment, it was clear a review of cleaning agents was needed to assess how to combat these surface pathogens. Cleaning is critical to keep hospital surfaces safe and preventing HCAI. However, protocols for cleaning are not standardised, and the quality and frequency of cleaning varies between hospitals. Overall, the lack of evidence-based guidance for clinical cleaning and wide array of cleaning options makes choosing a cleaning agent and delivering training a difficult undertaken, which is leading to poor compliance and cleaning failures throughout clinical settings.. The main issues were highlighted:

- Cleaning agents are tested under different conditions and are awarded different standards. A true measure of a cleaning agent should be challenged with both planktonic and carrier testing, as well as testing in the presence of soiling, to give the truest representation of how a cleaning agent may work for hospital surfaces.
- Different cleaning agents should be used for high, medium and low risk surfaces, though these classifications are perhaps outdated and need review. Regardless of cleaning agent chosen, physical cleaning must occur first, as disinfection cannot occur in the presence of debris or organic matter.

The review of cleaning agents showed how there were many different agents available for surface clinical cleaning, but perhaps most notably, the importance of ready to use surface wipes, which represent a fast and effective way of cleaning clinical environments. To assess their efficacy, a study of wipes used for clinical cleaning revealed the following:

- Ready-to-use (RTU) surface wipes are not created equal. Alcohol wipes dried out quickly and performed poorly when compared to other RTU wipes available for cleaning clinical surfaces.
- The gauze with no active ingredient performed well, and it was found that wipes have an optimum wetness, and wetter wipes allow better cleaning. However, care must be taken to ensure wipes are not too wet and as surface must be completely dry before use, and remaining wet for too long may hinder clinical practise.

- Wipes and liquid cleaning agents perform differently, and target organism, surface type and contact time should be considered carefully when selecting a cleaning agent.

With the efficacy of cleaning agents now put to the test, a ward sampling study was undertaken to put this into context and identify how well cleaning has been undertaken within a real clinical environment. The sampling study revealed the following:

- A multitude of factors have an impact on cleaning efficacy, such as personal perceptions of cleanability, perceptions of patient risk, surface material, staff groups delegated for cleaning, and population that interacts with the surface.
- Surfaces with lower CFU before cleaning became more contaminated after cleaning when compared with the surfaces with higher initial CFU

10.1.3 BEHAVIOUR AND TRAINING

With cleaning efficacy within a ward environment revealed, it was clear an intervention was required. The personal perceptions of surfaces had such an impact on cleaning, it was critical to first evaluate weaknesses in cleaning and develop a training intervention specifically based on this feedback. Following an audit re-audit study, surface wipe cleaning methods were inconsistent between all staff groups, and it was shown that nurses and cleaners had different understanding of cleaning and their role in cleaning, due to differences in training and backgrounds, and

therefore had different compliance to different components of the audit bundle. The main findings from the study were as follows:

- The education intervention had a small but effective positive impact on the targeted bundle components, identifying how the content and delivery of a training intervention is more important than the size of the intervention. This intervention represented a style and size of training that could be feasibly delivered, quickly and with few resources, within a busy ward setting.
- Personal judgement of a role and how cleaning should be undertaken can have an impact on how well cleaning is done, and this personal perception can be difficult to control.

To further understand how a pathogen may move and spread around a clinical environment, a DNA-based surrogate marker was used and inoculated within the clinical environment. This spread was tracked around a ward, and the results identified the following:

- Following the inoculation of a surrogate marker within the clinical environment, the surrogate spread rapidly when inoculated in the ward. Within 1 day, 41%, 30% and 41% of surfaces were contaminated with markers 1, 2 or 3.
- This spread highlights how any movement within the clinical environment, either by patients, visitors or staff, can facilitate the movement of an infectious agent.

- Some surfaces had simultaneous recovery of all three markers, which may represent an issue for potential gene transfer if the three markers represented different pathogenic organisms.
- Fast dissemination of the marker suggests poor hand hygiene and insufficient cleaning, as a single instance of proper cleaning or hand hygiene was shown to remove the marker.
- Movement of the marker from the isolation room into the bed bays represents an IPC risk, as isolation rooms are used for infected or colonised patients. This movement could put patients within the bays at risk.

10.2 LIMITATIONS OF THIS RESEARCH

No study is without limitations, and the limitations presented by this thesis are discussed in turn below. An important limitation to consider, for all work undertaken within the clinical environment (chapters 6, 7 and 9) is that all work was completed within a single paediatric hospital, covering two ward types (outpatients day unit and CICU). While all clinical environments are similar, they are not the same (different local policy, different building type and age, different patient subset, different services). Therefore findings from this study may not represent adult settings, or even other paediatric hospitals as a whole.

Furthermore, the work undertaken was short term; therefore the results represent a snapshot of the environment at that time. Changes within the ward may change the organisms present on the surfaces, movement of a surrogate or cleaning quality, such as outbreak, increased or decreased number of patients, change in patient turnover, outside training, season, or change of staff. Therefore, the results represent the conditions of that particular ward at that time period.

10.2.1 LITERATURE REVIEW LIMITATIONS

Care was taken to ensure an exhaustive list of key words and databases to search, though it is inevitable that some of the literature may be missed. The literature reviews were ongoing, though upon completion, were completed with the information currently available at the time. This may be out of date following new work. A detailed meta-analysis was not possible due to the broad subjects. The surface sampling literature review required a semi-systematic approach and unpublished or grey literature, that may contain valuable data, were not included.

10.2.2 Surface sampling limitations

- Pathogens were chosen to give a Gram-positive and Gram-negative representative, and to represent the species as a whole. However, these organisms may not cover all potential pathogens, and some may behave significantly differently.
- Sampling devices were tested across three different technicians due to volume of samples. This will lead to inevitable variations in technique, such as amount of pressure used when sampling. Careful supervision and training was used to reduce this variation to a minimum.
- Working with live organisms can also lead to natural variations in growth and recovery, of which are not preventable.
- Crowding of colonies on a plate can make some samples difficult to read, which can be mitigated by dilution of a sample where possible.

10.2.3 Ward sampling limitations

- Species testing was not undertaken and results were given in CFU. While this gives an indication as to the cleaning, risk cannot be determined. Therefore it was the assumption that any CFU recovered could, potentially, be a pathogenic organism.
- As with all surface sampling methodologies, the result is limited by the surface area sampled. This study, for the purposes of results, makes the assumption that the left side of the surfaces (sampled before cleaning) and the right side of the surfaces (sampled after cleaning) are representative of the entire surface, which was not tested within this study.

- Recovery with traditional microbiological methods may underestimate numbers of organisms on a surface, as stressed or damaged cells may not grow for plate count.
- Sampling was a snapshot of the environment at that time. Patient numbers, types of patient and season will likely change the environment, which would not be captured.

10.2.4 Observation study limitations

- The observations post-intervention were not repeated later within the year, therefore it cannot be determined how well the improvements from the training intervention would remain over time.
- The observation method considered and accounted for the Hawthorne effect, though this may not be ruled out entirely.
- Observations were not undertaken of specific staff before and after training, and instead captured cleaning efforts as a ward whole. Therefore individual improvements could not be determined.
- The staff captured before and after may differ due to absence, variation of staff and rota, and will inevitably have an impact on results.
- It was not possible to train every single staff member on the ward.
- The observations were a snapshot of that ward at that time period. Effort was taken to capture different ward times over different events (discharge, movement of patients, morning and evening handovers).
- Training intervention was based on the 'easiest' components of cleaning. Other cleaning components are important, such as contact time and order

of cleaning, though these were not used for the audit, as limited components could be chosen for audit.

- Observations method measured cleaning efficacy in relation to compliance to the cleaning bundle, as microbiological sampling and HCAI incidence data were not used as part of this study, if there had been an individual impact on HCAI incidence (which would be the goal standard of assessing result of a cleaning training intervention) then this was not highlighted. While improved cleaning undoubtedly has a positive impact on reducing environmental loading, there is no specific data from this study to state this.

10.2.5 **CaMV surrogate limitations**

- Due to practicality of sample collection and processing, a limited number of samples could be taken from the ward. These sampling sites were chosen strategically and to represent clinical significance, though it is important to note that more samples could give a clearer picture of how the surrogate disseminated within the ward. Additionally, samples were not taken outside the ward and from neighboring wards, therefore how far the surrogate travelled outside the ward could not be determined.
- Samples were taken with three technicians to ensure all samples could be taken within a timely manner. This may lead of variations in sampling, as some components of swab sampling cannot be standardised. How to sample and where to sample was identical, but components like pressure on the swab are hard to standardise.

- qPCR is a very sensitive method and can theoretically detect the presence of a single DNA copy. Swabs are the only method appropriate for recovering the oligonucleotide from the clinical surfaces, so processing losses are unavoidable. While qPCR is a very sensitive method, as well as assay and primer and run design, losses during swab sampling may cause underestimation during sampling. During the study, swab losses, on average, accounted for a loss of 5.50E+06 copies/µl.
- The results capture the movement of the ward at that particular time and no more.
- The use of three surrogate markers inoculated onto three surfaces allowed the identification of the movement of a potential infectious agent across these surfaces. More oligonucleotides could have been used on more surfaces, or different surfaces, for further assessment.
- The likelihood of infection/ infectious dose cannot be determined with the surrogate marker.
- The oligonucleotides make an excellent surrogate marker, though it is important to consider the limitations. The marker will be similar to an infectious material, though not the same, therefore the surrogate is not an exact picture of how an organism may move or persist in the environment. Furthermore, the surrogate did not have the capability to replicate, grow, or persist in biofilm.
- Many suggestions can be made from the results as to how the surrogate has moved between surfaces and the events that could have caused such

movement and deposition. However, these suggestions are cautious speculation only, and exact routine of movement can not be determine without a simultaneous observation study.

- It could not be determined if a surrogate was found on a surface specifically due to failed hand hygiene, or through inoculation from an intermediary source.

10.3 Future work

The work within this thesis could be expanded on in several ways:

The literature review highlighted the gaps within the literature regarding comprehensive analysis of surface sampling devices. While the work in chapter 3 worked to close this gap, further work is needed under a single, clear methodology to rigorously test all sampling devices across a range of surface materials, under different conditions such as humidity, temperature and organisms in biofilm. This will help give a clearer representation of how sampling devices may perform when recovering organisms from clinical environments.

The ward sampling was undertaken with contact plates, to give total CFU. It was understood that any colony could be a potential pathogen. Specific organism identification by MALDI-TOF or other molecular methods could explore the exact species present on the surfaces and therefore determine more specific risk. MALDI-TOF can also use subtyping to determine resistance markers, to identify important clinically significant pathogens such as MRSA or carbapenemase-producing *K. pneumoniae.* Sampling could also have been taken using selective media, to identify a range of select pathogens, such as MRSA.

The surrogate marker release could be undertaken within different types of clinical settings, such as adult hospitals or across a range of ward specialities. Additionally, running in parallel with an observation study and hand hygiene audit could closely reveal the exact movement of the marker. With a parallel observation study, specific deposition of the marker can be traced to the source, and important

areas of failure (hand hygiene failure, cleaning failure) can be identified, which can aid in future cleaning training and hand hygiene audit.

This study used three individual markers for the release. Future work with more markers could reveal closer interactions between different surface types. A larger number of samples could identify more movement and potential reservoirs for infection. Inclusion of surfaces from the wider hospital environment, such as waiting rooms outside of the ward, staff kitchens, staff rooms and canteens could explore if and how these non-clinical areas play a role in transmission.

Implementation of the audit re-audit cleaning training intervention in different wards could show how well the intervention works across different ward types. Frequent follow up in 3, 6 and 12 months with re-audit could reveal the longevity of the training, how frequently re-training should be implemented to give best results.

10.4 OVERALL THESIS THEMES

Across the 10 chapters, this thesis extensively reviews the literature on surface contaminants, how to find them and how to clean them. Gaps in the literature on surface sampling was identified (chapter 2), and addressed (chapter 3). Clinical surfaces were shown to be contaminated (chapter 1) therefore it was important to learn how to mitigate this surface contamination, assessed by a literature review of possible cleaning agents (chapter 4) efficacy testing of liquid agents and clinical surface wipes (chapter 5) and assessment of real cleaning within a ward (chapter 6). Cleaning was variable, and it was clear an intervention was needed (chapter 7) undertaken by an audit re-audit study following delivery of a specific training

package. To further assess how contamination might move around the clinical surface environment, a DNA marker study was used (chapters 8 and 9).

REFERENCES

[1] NHS. Healthcare associated infections. In: Improvement N, Ed.: NHS Improvement 2017.

[2] Gastmeier P From 'one size fits all' to personalized infection prevention. *J Hosp Infect* 2020; **104**: 256-60.

[3] Rutala WA, Weber DJ Surface disinfection: should we do it? *J Hosp Infect* 2001; **48**: S64-S8.

[4] Dharan SM, P.; Copin, P.; Bessmer, G.; Tschanz, B.; Pittet, D. Routine disinfection of patients' environmental surfaces. Myth or reality? *J Hosp Infect* 1999; **42**: 113-7.

[5] Malik RE, Cooper RA, Griffith CJ Use of audit tools to evaluate the efficacy of cleaning systems in hospitals. *Am J Infect Control* 2003; **31**: 181-7.

[6] Sherlock O, O'Connell N, Creamer E, Humphreys H Is it really clean? An evaluation of the efficacy of four methods for determining hospital cleanliness. *J Hosp Infect* 2009; **72**: 140-6.

[7] Gould JM, Coffin, S.E. *Principles and Practice of Pediatric Infectious Diseases,* Philadelphia: Elsevier 2018.

[8] Simoes AS, Couto I, Toscano C *et al.* Prevention and Control of Antimicrobial Resistant Healthcare-Associated Infections: The Microbiology Laboratory Rocks! *Front Microbiol* 2016; **7**.

[9] Pittet D, Harbarth S What techniques for diagnosis of ventilator-associated pneumonia? *Lancet* 1998; **352**: 83-4.

[10] Martin SJ, Yost RJ Infectious diseases in the critically ill patients. *J Pharm Pract* 2011; **24**: 35-43.

[11] Valencia R, Arroyo LA, Conde M *et al.* Nosocomial outbreak of infection with pan-drug-resistant Acinetobacter baumannii in a tertiary care university hospital. *Infect Control Hosp Epidemiol* 2009; **30**: 257-63.

[12] Weber DJ, Anderson D, Rutala WA The role of the surface environment in healthcare-associated infections. *Curr Opin Infect Dis* 2013; **26**: 338-44.

[13] Go ES, Urban C, Burns J *et al.* Clinical and molecular epidemiology of acinetobacter infections sensitive only to polymyxin B and sulbactam. *Lancet* 1994; **344**: 1329-32.

[14] Acar JF, Goldstein FW Consequences of increasing resistance to antimicrobial agents. *Clin Infect Dis* 1998; **27 Suppl 1**: S125-30.

[15] Aminov R History of antimicrobial drug discovery: Major classes and health impact. *Biochem Pharmacol* 2017; **133**: 4-19.

[16] Lewis K The Science of Antibiotic Discovery. *Cell* 2020; **181**: 29-45.

[17] El-Mahallawy HA, Hassan SS, El-Wakil M, Moneer MM Bacteremia due to ESKAPE pathogens: An emerging problem in cancer patients. *J Egypt Natl Canc Inst* 2016; **28**: 157-62.

[18] Klatte JM Pediatric Antimicrobial Stewardship Programs: Current Perspectives. *Pediatric Health Med Ther* 2020; **11**: 245-55.

[19] Nichols K, Stoffella S, Meyers R, Girotto J, Advocacy Committee for the Pediatric Pharmacy Advocacy G Pediatric Antimicrobial Stewardship Programs. *J Pediatr Pharmacol Ther* 2017; **22**: 77-80.

[20] Suleman L, Archer, D., Cochrane, C.A., Percival, S.L. *Chapter Ten - Healthcare-Associated Infections and Biofilms*: Academic Press 2014.

[21] Agency UHS. Staphylococcus aureus: guidance, data and analysis. In: The characteristics d, management, surveillance and epidemiology of Staphylococcus aureus., Ed.: UKHSA 2014.

[22] Ghasemzadeh-Moghaddam H, Ghaznavi-Rad E, Sekawi Z *et al.* Methicillin-susceptible Staphylococcus aureus from clinical and community sources are genetically diverse. *Int J Med Microbiol* 2011; **301**: 347-53.

[23] Rawlinson S, Ciric, L., Cloutman-Green, E Who Decides What's Relevant? Factors Driving Publication on Clinically Significant Organisms. *Infection Prevention in Practice* 2020; **2**.

[24] Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* 2015; **28**: 603-61.

[25] Vaishnavi C Translocation of gut flora and its role in sepsis. *Indian J Med Microbiol* 2013; **31**: 334-42.

[26] Ignacio A, Terra, F.F., Watanabe, I.K.M., Basso. P.J., Olsen, N., Camara, S. Microbiome and Metabolome in Diagnosis, Therapy, and other Strategic Applications. In: Faintuch J, Faintuch, S., Ed. *Role of the Microbiome in Intestinal Barrier Funection and Immune Defense*: Academic Press 2019; 127-38.

[27] Russotto V, Cortegiani A, Raineri SM, Giarratano A Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. *J Intensive Care* 2015; **3**: 54.

[28] Enoch DA, Cargill JS, Sismey A, Karas JA MRSA surveillance in a UK district hospital: measuring clinical isolates with MRSA is more useful than measuring MRSA bacteraemias. *J Hosp Infect* 2011; **79**: 287-91.

[29] Buchan BW, Graham MB, Lindmair-Snell J *et al.* The relevance of sink proximity to toilets on the detection of Klebsiella pneumoniae carbapenemase inside sink drains. *Am J Infect Control* 2019; **47**: 98-100.

[30] Kotsanas D, Wijesooriya WR, Korman TM *et al.* "Down the drain": carbapenem-resistant bacteria in intensive care unit patients and handwashing sinks. *Med J Aust* 2013; **198**: 267-9.

[31] Leitner E, Zarfel G, Luxner J *et al.* Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2-producing Klebsiella oxytoca on a hematology ward. *Antimicrob Agents Chemother* 2015; **59**: 714-6.

[32] PHE. Klebsiella species bacteraemia: annual data. In: Statistics N, Ed.: Public Health England 2018.

[33] Schultsz C, Geerlings S Plasmid-mediated resistance in Enterobacteriaceae: changing landscape and implications for therapy. *Drugs* 2012; **72**: 1-16.

[34] Carvalho I, Chenouf NS, Carvalho JA *et al.* Multidrug-resistant Klebsiella pneumoniae harboring extended spectrum beta-lactamase encoding genes isolated from human septicemias. *Plos One* 2021; **16**: e0250525.

[35] WHO. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. World Health Organisation 2017.

[36] Murdoch DR, Corey GR, Hoen B *et al.* Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study. *Arch Intern Med* 2009; **169**: 463-73.

[37] PHE. Enterococcus spp. bacteraemia: voluntary surveillance. In: analysis Ra, Ed.: Public Health England.

[38] Amyes SG Enterococci and streptococci. *Int J Antimicrob Agents* 2007; **29 Suppl 3**: S43-52.

[39] Fiore E, Van Tyne D, Gilmore MS Pathogenicity of Enterococci. *Microbiol Spectr* 2019; **7**.

[40] Lee K, Yoon SS Pseudomonas aeruginosa Biofilm, a Programmed Bacterial Life for Fitness. *J Microbiol Biotechnol* 2017; **27**: 1053-64.

[41] Hota S, Hirji Z, Stockton K *et al.* Outbreak of multidrug-resistant Pseudomonas aeruginosa colonization and infection secondary to imperfect intensive care unit room design. *Infect Control Hosp Epidemiol* 2009; **30**: 25-33.
[42] Weng MK, Brooks RB, Glowicz J *et al.* Outbreak investigation of Pseudomonas aeruginosa infections in a neonatal intensive care unit. *Am J Infect Control* 2019; **47**: 1148-50.
[43] Engelhart S, Krizek L, Glasmacher A, Fischnaller E, Marklein G, Exner M Pseudomonas aeruginosa outbreak in a haematology-oncology unit associated with contaminated surface cleaning equipment. *J Hosp Infect* 2002; **52**: 93-8.
[44] Catho G, Martischang R, Boroli F *et al.* Outbreak of Pseudomonas aeruginosa producing VIM carbapenemase in an intensive care unit and its termination by implementation of waterless patient care. *Crit Care* 2021; **25**: 301.
[45] Enoch DA, Kuzhively J, Sismey A, Grynik A, Karas JA Pseudomonas Aeruginosa Bacteraemia in Two UK District Hospitals. *Infect Dis Rep* 2013; **5**: e4.
[46] England PH. Pseudomonas aeruginosa: guidance, data and analysis. Public Health England 2014.
[47] Madani K Dr. Hans Christian Jaochim Gram: inventor of the Gram stain. *Primary Care Update for OB/GYNS* 2003; **10**: 235-7.
[48] Breijyeh Z, Jubeh B, Karaman R Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules* 2020; **25**.
[49] Friedline A, Zachariah M, Middaugh A *et al.* Sterilization of hydrogen peroxide resistant bacterial spores with stabilized chlorine dioxide. *AMB Express* 2015; **5**: 24.
[50] Miller SI Antibiotic Resistance and Regulation of the Gram-Negative Bacterial Outer Membrane Barrier by Host Innate Immune Molecules. *mBio* 2016; **7**.
[51] Gupta V, Datta P Next-generation strategy for treating drug resistant bacteria: Antibiotic hybrids. *Indian J Med Res* 2019; **149**: 97-106.
[52] Burnett-Boothroyd SC, McCarthy, B.J. Textiles for Hygiene and Infection Control. *Antimicrobial treatments of textiles for hygiene and infection control applications: an industrial perspective*: Woodhead Publishing 2011; 196-209.
[53] Shales DM. In: Moo-Young M, Ed. *Comprehensive Biotechnology,* Second Edition edn.: Academic Press 2011; 243-54.
[54] Spellberg B, Gilbert DN The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. *Clin Infect Dis* 2014; **59 Suppl 2**: S71-5.
[55] Ventola CL The antibiotic resistance crisis: part 1: causes and threats. *P T* 2015; **40**: 277-83.
[56] Rossato L, Negrao FJ, Simionatto S Could the COVID-19 pandemic aggravate antimicrobial resistance? *Am J Infect Control* 2020; **48**: 1129-30.
[57] Huttner BD, Catho G, Pano-Pardo JR, Pulcini C, Schouten J COVID-19: don't neglect antimicrobial stewardship principles! *Clin Microbiol Infect* 2020; **26**: 808-10.
[58] Gottig S, Gruber TM, Higgins PG, Wachsmuth M, Seifert H, Kempf VA Detection of pan drug-resistant Acinetobacter baumannii in Germany. *The Journal of antimicrobial chemotherapy* 2014; **69**: 2578-9.
[59] Bigger JW TREATMENT OF STAPHYLOCOCCAL INFECTIONS WITH PENICILLIN BY INTERMITTENT STERILISATION. *The Lancet* 1944; **244**: 497-500.

[60] Lewis K Persister cells, dormancy and infectious disease. *Nat Rev Microbiol* 2007; **5**: 48-56.
[61] Reygaert WC An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol* 2018; **4**: 482-501.
[62] Fernandes S, Gomes IB, Sousa SF, Simoes M Antimicrobial Susceptibility of Persister Biofilm Cells of Bacillus cereus and Pseudomonas fluorescens. *Microorganisms* 2022; **10**.
[63] Siddiqui S. Chapter 14 - Resistance in Pathogenic Microorganisms. In: Gupta VK, Pandey, A., Ed. *New and Future Developments in Microbial Biotechnology and Bioengineering*: Elsevier 2019; 183-91.
[64] Olivares J, Bernardini A, Garcia-Leon G, Corona F, M BS, Martinez JL The intrinsic resistome of bacterial pathogens. *Front Microbiol* 2013; **4**: 103.
[65] Bernardini A, Cuesta T, Tomas A, Bengoechea JA, Martinez JL, Sanchez MB The intrinsic resistome of Klebsiella pneumoniae. *Int J Antimicrob Agents* 2019; **53**: 29-33.
[66] CDC. How Antibiotic Resistance Happens. In: National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) DoH, Ed.: CDC 2021.
[67] Tenover FC Mechanisms of antimicrobial resistance in bacteria. *Am J Infect Control* 2006; **34**: S3-10; discussion S64-73.
[68] Opal SM, Pop-Vicas, A. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. In: Bennett JE, Dolin, R., Blaser, M.J, Ed. *18 - Molecular Mechanisms of Antibiotic Resistance in Bacteria*: W.B Saunders 2015; 235-51.
[69] Ciofu O, Moser C, Jensen PO, Hoiby N Tolerance and resistance of microbial biofilms. *Nat Rev Microbiol* 2022; doi 10.1038/s41579-022-00682-4.
[70] Pan YP, Xu YH, Wang ZX, Fang YP, Shen JL Overexpression of MexAB-OprM efflux pump in carbapenem-resistant Pseudomonas aeruginosa. *Arch Microbiol* 2016; **198**: 565-71.
[71] Travisano M. Bacterial Genetics. In: Levin SA, Ed. *Encyclopedia of Biodiversity*: Elsevier 2001; 339-50.
[72] McManus MC Mechanisms of bacterial resistance to antimicrobial agents. *Am J Health Syst Pharm* 1997; **54**: 1420-33; quiz 44-6.
[73] Finley RL, Collignon P, Larsson DG *et al.* The scourge of antibiotic resistance: the important role of the environment. *Clin Infect Dis* 2013; **57**: 704-10.
[74] Khanna NR, Gerriets V. Beta Lactamase Inhibitors. *StatPearls* Treasure Island (FL) 2022.
[75] Bush K, Bradford PA beta-Lactams and beta-Lactamase Inhibitors: An Overview. *Cold Spring Harb Perspect Med* 2016; **6**.
[76] Stryjewski ME, Corey GR Methicillin-resistant Staphylococcus aureus: an evolving pathogen. *Clin Infect Dis* 2014; **58 Suppl 1**: S10-9.
[77] Hanssen AM, Ericson Sollid JU SCCmec in staphylococci: genes on the move. *FEMS Immunol Med Microbiol* 2006; **46**: 8-20.
[78] Tsubakishita S, Kuwahara-Arai K, Sasaki T, Hiramatsu K Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. *Antimicrob Agents Chemother* 2010; **54**: 4352-9.
[79] Acharya Y, Dhanda G, Sarkar P, Haldar J Pursuit of next-generation glycopeptides: a journey with vancomycin. *Chem Commun (Camb)* 2022; **58**: 1881-97.
[80] Alby M, Miller, M.B. Principles and Practice of Pediatric Infectious Diseases. In: Long SS, Prober, C.G., Fischer, M., Ed. *Mechanisms and Detection of Antimicrobial Resistance,* Fifth edn.: Elsevier 2018; 1467-78.
[81] Rubinstein EPL-W. *Infectious Diseases,* Fourth Edition edn. 2017.

[82] Drlica K, Hiasa H, Kerns R, Malik M, Mustaev A, Zhao X Quinolones: action and resistance updated. *Curr Top Med Chem* 2009; **9**: 981-98.
[83] Bal AM Macrolide Antibiotics. *Reference Module in Biomedical Sciences* 2021.
[84] Malmir S, Bahreinian, M., Yeganeh, S.Z., Mirnejad, R., Moghaddam, M.M., Saberi, F. Molecular Mechanisms of Resistance to Conventional Antibiotics in Bacteria. *International Journal of Medical Reviews* 2018; **5**: 118-29.
[85] Vazquez-Laslop N, Mankin AS How Macrolide Antibiotics Work. *Trends Biochem Sci* 2018; **43**: 668-84.
[86] Munita JM, Arias CA Mechanisms of Antibiotic Resistance. *Microbiol Spectr* 2016; **4**.
[87] Spaulding EH. *Chemical disinfection of medical and surgical materials,* Philadephia: Lea & Febiger 1968.
[88] McDonnell G, Burke P Disinfection: is it time to reconsider Spaulding? *J Hosp Infect* 2011; **78**: 163-70.
[89] Vasickova P, Pavlik, I., Verani, M., Carducci, A Issues Concerning Survival of Viruses on Surfaces. *Food Environ Virol* 2020; **2**: 24-34.
[90] Otter JA, Yezli S, French GL The Role Played by Contaminated Surfaces in the Transmission of Nosocomial Pathogens. *Infect Cont Hosp Ep* 2011; **32**: 687-99.
[91] Kohli R. Developments in Surface Contamination and Cleaning. In: Kohli R, Mittal, K.L., Ed. *Methods of Cleaning and Cleanliness Verification*: William Andrew Publishing 2013; 139-61.
[92] Rawlinson S, Cloutman-Green E, Asadi F, Ciric L Surface sampling within a pediatric ward-how multiple factors affect cleaning efficacy. *Am J Infect Control* 2020; **48**: 740-5.
[93] Knelson LP, Williams DA, Gergen MF *et al.* A Comparison of Environmental Contamination by Patients Infected or Colonized with Methicillin-Resistant Staphylococcus aureus or Vancomycin-Resistant Enterococci: A Multicenter Study. *Infect Cont Hosp Ep* 2014; **35**: 872-5.
[94] Hota B Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? *Clin Infect Dis* 2004; **39**: 1182-9.
[95] Boyce JM Environmental contamination makes an important contribution to hospital infection. *J Hosp Infect* 2007; **65 Suppl 2**: 50-4.
[96] Huang SS, Datta R, Platt R Risk of acquiring antibiotic-resistant bacteria from prior room occupants. *Arch Intern Med* 2006; **166**: 1945-51.
[97] Arzilli G, Scardina G, Casigliani V *et al.* Screening for antimicrobial-resistant Gram-negative bacteria in hospitalised patients, and risk of progression from colonisation to infection: Systematic review. *J Infect* 2022; **84**: 119-30.
[98] Hong KB, Oh HS, Song JS *et al.* Investigation and control of an outbreak of imipenem-resistant Acinetobacter baumannii Infection in a Pediatric Intensive Care Unit. *Pediatr Infect Dis J* 2012; **31**: 685-90.
[99] Taneja N, Emmanuel R, Singh M, Sharma M Hospital environment contamination with Gram-negative bacteria is as common and important as that with Gram-positive bacteria in an overloaded tertiary care hospital in India. *J Hosp Infect* 2005; **59**: 164-5.
[100] Houldcroft CJ, Roy S, Morfopoulou S *et al.* Use of Whole-Genome Sequencing of Adenovirus in Immunocompromised Pediatric Patients to Identify Nosocomial Transmission and Mixed-Genotype Infection. *J Infect Dis* 2018; **218**: 1261-71.
[101] Nutman A, Marchaim D How to: molecular investigation of a hospital outbreak. *Clin Microbiol Infect* 2019; **25**: 688-95.

[102] Rump B, Cornelis C, Woonink F, J VANS, Verweij M, Hulscher M Using typing techniques in a specific outbreak: the ethical reflection of public health professionals. *Epidemiol Infect* 2017; **145**: 1431-6.
[103] Lee LM, Heilig, C.M., White, A. Ethical justification for conducting public health surveillance without patient consent. *Am J Public Health* 2012; **102**: 38-44.
[104] Mody L, Washer LL, Kaye KS *et al.* Multidrug-resistant Organisms in Hospitals: What Is on Patient Hands and in Their Rooms? *Clin Infect Dis* 2019; **69**: 1837-44.
[105] Bonadonna L, Briancesco, R., Coccia, A.M. Analysis of Microorganisms in Hospital Environments and Potential Risks. *Indoor Air Quality in Healthcare Facilities* 2017; doi https://doi.org/10.1007/978-3-319-49160-8_5: 53-62.
[106] Chia PY, Sengupta S, Kukreja A, S SLP, Ng OT, Marimuthu K The role of hospital environment in transmissions of multidrug-resistant gram-negative organisms. *Antimicrob Resist Infect Control* 2020; **9**: 29.
[107] Oliver JD The viable but nonculturable state in bacteria. *Journal of microbiology (Seoul, Korea)* 2005; **43 Spec No**: 93-100.
[108] Stewart EJ Growing unculturable bacteria. *J Bacteriol* 2012; **194**: 4151-60.
[109] Staley JT, Konopka A Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu Rev Microbiol* 1985; **39**: 321-46.
[110] Arnold JW, Bailey GW Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: scanning electron and atomic force microscopy study. *Poult Sci* 2000; **79**: 1839-45.
[111] Klassert TE, Zubiria-Barrera C, Neubert R *et al.* Comparative analysis of surface sanitization protocols on the bacterial community structures in the hospital environment. *Clin Microbiol Infect* 2022; doi 10.1016/j.cmi.2022.02.032.
[112] Murray AN Food Safety Assurance Systems: Hygienic Design of Equipment. *Reference Module in Food Sciences* 2014; **4**: 181-8.
[113] Meireles A, Simoes, M. Nanotechnology in the Agri-Food Industry. In: Grumezescu AM, Ed. *Food Presevation*: Academic Press 2017; 167-95.
[114] Langard S, Costa, M. Handbook on the Toxicology of Metals. In: Nordberg GF, Fowler, B.A., Nordberg, M., Friberg, L.T., Ed. *Chromium,* Third edn.: Academic Press 2007; 478-510.
[115] Lo KH, Shek, C.H., Lai, J.K.L Recent developments in stainless steels. *Materials Science and Engineering: R: Reports* 2009; **65**: 39-104.
[116] Palmer J, Flint S, Brooks J Bacterial cell attachment, the beginning of a biofilm. *J Ind Microbiol Biotechnol* 2007; **34**: 577-88.
[117] Flint SH, Brooks JD, Bremer PJ The influence of cell surface properties of thermophilic streptococci on attachment to stainless steel. *J Appl Microbiol* 1997; **83**: 508-17.
[118] Ebbing D, Gammon, S.D. *General Chemistry,* Eleventh edn. Boston: Cengage Learning 2016.
[119] Takoi H, Fujita K, Hyodo H *et al.* Acinetobacter baumannii can be transferred from contaminated nitrile examination gloves to polypropylene plastic surfaces. *Am J Infect Control* 2019; **47**: 1171-5.
[120] Beneš P, Paulenová, M. Surface charge and adsorption properties of polyethylene in aqueous solutions of inorganic electrolytes. *Kolloid-ZuZPolymere* 1973; **251**: 766-71.
[121] Monfort E, Mezquita, A., Vaquer, E., Celades, I., Sanfelix, V., Escrig, A. Comprehensive Materials Processing. In: Hashmi S, Batalha, G.F., Van Tyne, C.J., Yilbas, B., Ed. *Materials Science and Materials Engineering*: Elsevier 2014; 71-102.

[122] Best EL, Fawley WN, Parnell P, Wilcox MH The Potential for Airborne Dispersal of Clostridium difficile from Symptomatic Patients. *Clin Infect Dis* 2010; **50**: 1450-7.
[123] Moore G, Muzslay M, Wilson APR The Type, Level, and Distribution of Microorganisms within the Ward Environment: A Zonal Analysis of an Intensive Care Unit and a Gastrointestinal Surgical Ward. *Infect Cont Hosp Ep* 2013; **34**: 500-6.
[124] Lerner A, Adler A, Abu-Hanna J, Meitus I, Navon-Venezia S, Carmeli Y Environmental Contamination by Carbapenem-Resistant Enterobacteriaceae. *J Clin Microbiol* 2013; **51**: 177-81.
[125] Kim KH, Fekety R, Batts DH *et al.* Isolation of Clostridium difficile from the environment and contacts of patients with antibiotic-associated colitis. *J Infect Dis* 1981; **143**: 42-50.
[126] Adams CE, Smith J, Watson V, Robertson C, Dancer SJ Examining the association between surface bioburden and frequently touched sites in intensive care. *J Hosp Infect* 2017; **95**: 76-80.
[127] Sexton T, Clarke P, O'Neill E, Dillane T, Humphreys H Environmental reservoirs of methicillin-resistant Staphylococcus aureus in isolation rooms: correlation with patient isolates and implications for hospital hygiene. *J Hosp Infect* 2006; **62**: 187-94.
[128] Boyce JM, Potter-Bynoe G, Chenevert C, King T Environmental contamination due to methicillin-resistant Staphylococcus aureus: Possible infection control implications. *Infect Cont Hosp Ep* 1997; **18**: 622-7.
[129] Nutman A, Lerner A, Schwartz D, Carmeli Y Evaluation of carriage and environmental contamination by carbapenem-resistant Acinetobacter baumannii. *Clin Microbiol Infec* 2016; **22**.
[130] Hayden MK, Bonten MJ, Blom DW, Lyle EA, van de Vijver DA, Weinstein RA Reduction in acquisition of vancomycin-resistant enterococcus after enforcement of routine environmental cleaning measures. *Clin Infect Dis* 2006; **42**: 1552-60.
[131] Hota B, Blom DW, Lyle EA, Weinstein RA, Hayden MK Interventional evaluation of environmental contamination by vancomycin-resistant enterococci: failure of personnel, product, or procedure? *J Hosp Infect* 2009; **71**: 123-31.
[132] Dancer SJ, White LF, Lamb J, Girvan EK, Robertson C Measuring the effect of enhanced cleaning in a UK hospital: a prospective cross-over study. *BMC Med* 2009; **7**: 28.
[133] Eckstein BC, Adams DA, Eckstein EC *et al.* Reduction of Clostridium Difficile and vancomycin-resistant Enterococcus contamination of environmental surfaces after an intervention to improve cleaning methods. *Bmc Infect Dis* 2007; **7**: 61.
[134] Mitchell BG, Dancer SJ, Anderson M, Dehn E Risk of organism acquisition from prior room occupants: a systematic review and meta-analysis. *J Hosp Infect* 2015; **91**: 211-7.
[135] Mathur P Hand hygiene: back to the basics of infection control. *Indian J Med Res* 2011; **134**: 611-20.
[136] Boyce JM, Pittet D, Healthcare Infection Control Practices Advisory Committee. Society for Healthcare Epidemiology of America. Association for Professionals in Infection Control. Infectious Diseases Society of America. Hand Hygiene Task F Guideline for Hand Hygiene in Health-Care Settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. *Infect Control Hosp Epidemiol* 2002; **23**: S3-40.

[137] Larson E A causal link between handwashing and risk of infection? Examination of the evidence. *Infect Cont Hosp Ep* 1988; **9**: 28-36.
[138] Larson E Skin hygiene and infection prevention: more of the same or different approaches? *Clin Infect Dis* 1999; **29**: 1287-94.
[139] Rawlinson S, Ciric L, Cloutman-Green E How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence. *J Hosp Infect* 2019; doi 10.1016/j.jhin.2019.07.015.
[140] Deshpande A, Dunn AN, Fox J *et al.* Monitoring the effectiveness of daily cleaning practices in an intensive care unit (ICU) setting using an adenosine triphosphate (ATP) bioluminescence assay. *Am J Infect Control* 2020; **48**: 757-60.
[141] Trsan M, Vehovc M, Seme K, Srcic S Evaluation of ATP bioluminescence for monitoring surface hygiene in a hospital pharmacy cleanroom. *J Microbiol Methods* 2020; **168**: 105785.
[142] Mitchell BG, Wilson, F., McGregor, A., Dancer, S. Methods to evaluate environmental cleanliness in healthcare facilities. *Healthcare Infection* 2013; **18**: 23-30.
[143] Blaiotta G, Fusco V, Ercolini D, Pepe O, Coppola S Diversity of Staphylococcus species strains based on partial kat (catalase) gene sequences and design of a PCR-restriction fragment length polymorphism assay for identification and differentiation of coagulase-positive species (S. aureus, S. delphini, S. hyicus, S. intermedius, S. pseudintermedius, and S. schleiferi subsp. coagulans). *J Clin Microbiol* 2010; **48**: 192-201.
[144] Yamamoto Y PCR in diagnosis of infection: detection of bacteria in cerebrospinal fluids. *Clin Diagn Lab Immunol* 2002; **9**: 508-14.
[145] Wade W Unculturable bacteria--the uncharacterized organisms that cause oral infections. *J R Soc Med* 2002; **95**: 81-3.
[146] Kramer A, Schwebke, I., Kampf, G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *Bmc Infect Dis* 2006; **6**.
[147] Kussell E, Kishony R, Balaban NQ, Leibler S Bacterial persistence: a model of survival in changing environments. *Genetics* 2005; **169**: 1807-14.
[148] Lopez D, Vlamakis H, Kolter R Biofilms. *Cold Spring Harb Perspect Biol* 2010; **2**: a000398.
[149] Kostakioti M, Hadjifrangiskou M, Hultgren SJ Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb Perspect Med* 2013; **3**: a010306.
[150] Jha PK, Dallagi, H., Richard, E., Benezech, T., Faille, C. Formation and resistance to cleaning of biofilms at air-liquid-wall interface. Influence of bacterial strain and material. *Food Control* 2020; **118**.
[151] Ledwoch K, Magoga M, Williams D, Fabbri S, Walsh J, Maillard JY Is a reduction in viability enough to determine biofilm susceptibility to a biocide? *Infect Control Hosp Epidemiol* 2021; **42**: 1486-92.
[152] Garrett TR, Bhakoo, M., Zhang, Z Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science* 2008; **18**: 1049-56.
[153] Ferreira AM, Andrade, D., Rigotti, M.A., Ferreira, M.V.F. Condition of Cleanliness of Surfaces Close to Patients in an Intensive Care Unit. *Rev Lat-Am Enferm* 2011; **19**: 557-64.
[154] Griffith CJ, Cooper RA, Gilmore J, Davies C, Lewis M An evaluation of hospital cleaning regimes and standards. *J Hosp Infect* 2000; **45**: 19-28.
[155] Otter JA, Mutters NT, Tacconelli E, Gikas A, Holmes AH Controversies in guidelines for the control of multidrug-resistant Gram-negative bacteria in EU countries. *Clin Microbiol Infect* 2015; **21**: 1057-66.
[156] Mota JD, Boue, G., Prevost, H., Maillet, A., Jaffres, E., Maignien, T., Arnich, N., Sanaa, M., Federighi, M Environmental monitoring program to support

food microbiological safety and quality in food industries: A scoping review of the research and guidelines. *Food Control* 2021; **130**.
[157] Mark BL, McKenna, S.A., Khajehpour, M. Comprehensive Biotechnology. In: Moo-Young M, Ed. *Protein Structural Analysis,* Third edn.: Pergamon 2011; 116-30.
[158] Dewangan A, Gaikwad U Comparative evaluation of a novel fluorescent marker and environmental surface cultures to assess the efficacy of environmental cleaning practices at a tertiary care hospital. *J Hosp Infect* 2020; **104**: 261-8.
[159] Gillespie E, Wright PL, Snook K *et al.* The role of ultraviolet marker assessments in demonstrating cleaning efficacy. *Am J Infect Control* 2015; **43**: 1347-9.
[160] Knowles JR Enzyme-catalyzed phosphoryl transfer reactions. *Annu Rev Biochem* 1980; **49**: 877-919.
[161] Mitchell BG, McGhie A, Whiteley G *et al.* Evaluating bio-burden of frequently touched surfaces using Adenosine Triphosphate bioluminescence (ATP): Results from the Researching Effective Approaches to Cleaning in Hospitals (REACH) trial. *Infect Dis Health* 2020; **25**: 168-74.
[162] Raghavendra P, Pullaiah, T. Pathogen Identification Using Novel Sequencing Methods. In: Raghavendra P, Pullaiah, T., Ed. *Advances in Cell and Molecular Diagnostic*: Academic Press 2018; 161-202.
[163] Omidbakhsh N, Ahmadpour F, Kenny N How reliable are ATP bioluminescence meters in assessing decontamination of environmental surfaces in healthcare settings? *Plos One* 2014; **9**: e99951.
[164] Gibbs SG, Sayles H, Colbert EM, Hewlett A, Chaika O, Smith PW Evaluation of the relationship between the Adenosine Triphosphate (ATP) bioluminescence assay and the presence of Bacillus anthracis spores and vegetative cells. *Int J Environ Res Public Health* 2014; **11**: 5708-19.
[165] van Arkel A, Willemsen I, Kluytmans J The correlation between ATP measurement and microbial contamination of inanimate surfaces. *Antimicrob Resist Infect Control* 2021; **10**: 116.
[166] Getchellwhite SI, Donowitz LG, Groschel DHM The Inanimate Environment of an Intensive-Care Unit as a Potential Source of Nosocomial Bacteria - Evidence for Long Survival of Acinetobacter-Calcoaceticus. *Infect Cont Hosp Ep* 1989; **10**: 402-7.
[167] Buttner MP, Cruz P, Stetzenbach LD, Cronin T Evaluation of two surface sampling methods for detection of Erwinia herbicola on a variety of materials by culture and quantitative PCR. *Appl Environ Microbiol* 2007; **73**: 3505-10.
[168] Rose L, Jensen B, Peterson A, Banerjee SN, Srduino MJ Swab materials and Bacillus anthracis spore recovery from nonporous surfaces. *Emerg Infect Dis* 2004; **10**: 1023-9.
[169] Landers TF, Hoet A, Wittum TE Swab Type, Moistening, and Preenrichment for Staphylococcus aureus on Environmental Surfaces. *J Clin Microbiol* 2010; **48**: 2235-6.
[170] Dolan A, Bartlett M, McEntee B, Creamer E, Humphreys H Evaluation of different methods to recover meticillin-resistant Staphylococcus aureus from hospital environmental surfaces. *J Hosp Infect* 2011; **79**: 227-30.
[171] Assadian O, Harbarth S, Vos M, Knobloch JK, Asensio A, Widmer AF Practical recommendations for routine cleaning and disinfection procedures in healthcare institutions: a narrative review. *J Hosp Infect* 2021; **113**: 104-14.
[172] Lewis T, Griffith C, Gallo M, Weinbren M A modified ATP benchmark for evaluating the cleaning of some hospital environmental surfaces. *J Hosp Infect* 2008; **69**: 156-63.

[173] Trsan M, Seme K, Srcic S The environmental monitoring in hospital pharmacy cleanroom and microbiota catalogue preparation. *Saudi Pharm J* 2019; **27**: 455-62.
[174] Claro T, O'Reilly M, Daniels S, Humphreys H Surface microbial contamination in hospitals: A pilot study on methods of sampling and the use of proposed microbiologic standards. *Am J Infect Control* 2015; **43**: 1000-2.
[175] Konieczka P. Comprehensive Sampling and Sample Preparation. In: Pawliszyn J, Ed. *Comprehensive Sampling and Sample Preparation*: Academic Press 2012; 699-711.
[176] Sutton S Accuracy of Plate Counts. *Journal of Validation of Technology* 2011; **17**: 42-6.
[177] Moore G, Griffith C A comparison of surface sampling methods for detecting coliforms on food contact surfaces. *Food Microbiol* 2002; **19**: 65-73.
[178] Buttner MP, Cruz-Perez P, Stetzenbach LD Enhanced detection of surface-associated bacteria in indoor environments by quantitative PCR. *Appl Environ Microbiol* 2001; **67**: 2564-70.
[179] Cangelosi GA, Meschke JS Dead or alive: molecular assessment of microbial viability. *Appl Environ Microbiol* 2014; **80**: 5884-91.
[180] Wan GH, Huang CG, Chung FF, Lin TY, Tsao KC, Huang YC Detection of Common Respiratory Viruses and Mycoplasma pneumoniae in Patient-Occupied Rooms in Pediatric Wards. *Medicine* 2016; **95**.
[181] Reha-Krantz LJ, Zhang, L. Primer. In: Maloy S, Hughes, K., Ed. *Brenner's Encyclopedia of Genetics,* Second edn.: Academic Press 2013; 454-6.
[182] Wages JM. Polymerase Chain Reaction. In: Worsfold P, Townshend, A., Poole, C, Ed. *Encyclopedia of Analytical Science,* 2nd edn.: Elsevier 2005; 243-50.
[183] Valones MA, Guimaraes RL, Brandao LA, de Souza PR, de Albuquerque Tavares Carvalho A, Crovela S Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. *Braz J Microbiol* 2009; **40**: 1-11.
[184] Maga G. DNA Polymerases. In: Maga G, Ed. *Reference Module in Biomedical Science*: Elsevier 2019; 376-8.
[185] NCBI. Polymerase Chain Reaction. *National Library of Medicine*: NCBI.
[186] Rawlinson S, Ciric, L., Cloutman-Green, E COVID-19 pandemic - let's not forget surfaces *J Hosp Infect* 2020; doi,https://doi.org/10.1016/j.jhin.2020.05.022.
[187] Krishnamurthy T, Ross, P.L., Rajamani, U. Detection of pathogenic and non-pathogenic bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry* 1996; **10**: 883-8.
[188] Carbonnelle E, Mesquita C, Bille E *et al.* MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin Biochem* 2011; **44**: 104-9.
[189] Ashfaq MY, Da'na DA, Al-Ghouti MA Application of MALDI-TOF MS for identification of environmental bacteria: A review. *J Environ Manage* 2022; **305**: 114359.
[190] Hrabak J, Chudackova E, Walkova R Matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. *Clin Microbiol Rev* 2013; **26**: 103-14.
[191] Mitchell BG, Farrington, A., Allen, M., Gardner, A., Hall, L., Barnett, A.G., Halton, K., Page, K., Dancer, S.J., Riley, T.V., Gericke, C.A., Paterson, D.L., Graves, N. Variation in hospital cleaning practice and process in Australian

hospitals: A structured mapping exercise. *Infection, Disease & Health* 2017; **22**: 195-202.
[192] Rutala WA, Weber DJ Disinfection and sterilization: an overview. *Am J Infect Control* 2013; **41**: S2-5.
[193] Kenters N, Huijskens EGW, de Wit SCJ *et al.* Effectiveness of various cleaning and disinfectant products on Clostridium difficile spores of PCR ribotypes 010, 014 and 027. *Antimicrob Resist Infect Control* 2017; **6**: 54.
[194] European Chemicals Agency. Overview of (EN) standards, test conditions, and pass criteria. In: group Bew, Ed.: ECHA 2017.
[195] Robertson A, Barrell M, Maillard JY Combining detergent/disinfectant with microfibre material provides a better control of microbial contaminants on surfaces than the use of water alone. *J Hosp Infect* 2019; **103**: e101-e4.
[196] Doll M, Stevens M, Bearman G Environmental cleaning and disinfection of patient areas. *Int J Infect Dis* 2018; **67**: 52-7.
[197] Mitchell BG, Hall L, White N *et al.* An environmental cleaning bundle and health-care-associated infections in hospitals (REACH): a multicentre, randomised trial. *Lancet Infect Dis* 2019; **19**: 410-8.
[198] Allen M, Hall L, Halton K, Graves N Improving hospital environmental hygiene with the use of a targeted multi-modal bundle strategy. *Infecti, Dis Health* 2018; **23**: 107-13.
[199] Carling PC, Parry MF, Bruno-Murtha LA, Dick B Improving environmental hygiene in 27 intensive care units to decrease multidrug-resistant bacterial transmission. *Crit Care Med* 2010; **38**: 1054-9.
[200] Rawlinson S, Faiza, A., Saraqi, H., Childs, B., Ciric, L., Cloutman-Green, E Does size matter? the impact of a small but targeted cleaning training intervention within a paediatric ward. *Infection Prevention in Practice* 2020; doi https://doi.org/10.1016/j.infpip.2020.100083.
[201] Kenters N, Gottlieb T, Hopman J *et al.* An international survey of cleaning and disinfection practices in the healthcare environment. *J Hosp Infect* 2018; **100**: 236-41.
[202] Hopmans TEM, Smid EA, Wille JC *et al.* Trends in prevalence of healthcare-associated infections and antimicrobial use in hospitals in the Netherlands: 10 years of national point-prevalence surveys. *J Hosp Infect* 2020; **104**: 181-7.
[203] Haque M, Sartelli M, McKimm J, Abu Bakar M Health care-associated infections - an overview. *Infect Drug Resist* 2018; **11**: 2321-33.
[204] Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E Role of hospital surfaces in the transmission of emerging health care-associated pathogens: Norovirus, Clostridium difficile, and Acinetobacter species. *Am J Infect Control* 2010; **38**: S25-S33.
[205] Otter JA, Yezli S, Salkeld JA, French GL Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. *Am J Infect Control* 2013; **41**: S6-11.
[206] Marchetti A, Rossiter R Economic burden of healthcare-associated infection in US acute care hospitals: societal perspective. *J Med Econ* 2013; **16**: 1399-404.
[207] Plowman R The socioeconomic burden of hospital acquired infection. *Euro Surveill* 2000; **5**: 49-50.
[208] Dancer SJ The role of environmental cleaning in the control of hospital-acquired infection. *J Hosp Infect* 2009; **73**: 378-85.
[209] Martinez JA, Ruthazer R, Hansjosten K, Barefoot L, Snydman DR Role of environmental contamination as a risk factor for acquisition of vancomycin-

resistant enterococci in patients treated in a medical intensive care unit. *Arch Intern Med* 2003; **163**: 1905-12.
[210] Arnold C Rethinking sterile: the hospital microbiome. *Environ Health Perspect* 2014; **122**: A182-7.
[211] Rampelotto PH, Sereia AFR, de Oliveira LFV, Margis R Exploring the Hospital Microbiome by High-Resolution 16S rRNA Profiling. *International journal of molecular sciences* 2019; **20**.
[212] Dancer SJ How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals. *J Hosp Infect* 2004; **56**: 10-5.
[213] Griffith CJ, Malik R, Cooper RA, Looker N, Michaels B Environmental surface cleanliness and the potential for contamination during handwashing. *Am J Infect Control* 2003; **31**: 93-6.
[214] Liberati A, Altman DG, Tetzlaff J *et al.* The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. *Ann Intern Med* 2009; **151**: W65-W94.
[215] Chen LF, Knelson LP, Gergen MF *et al.* A prospective study of transmission of Multidrug-Resistant Organisms (MDROs) between environmental sites and hospitalized patientsthe TransFER study. *Infect Cont Hosp Ep* 2019; **40**: 47-52.
[216] Dallolio L, Raggi A, Sanna T *et al.* Surveillance of Environmental and Procedural Measures of Infection Control in the Operating Theatre Setting. *Int J Env Res Pub He* 2018; **15**.
[217] Muzslay M, Moore G, Alhussaini N, Wilson APR ESBL-producing Gram-negative organisms in the healthcare environment as a source of genetic material for resistance in human infections. *J Hosp Infect* 2017; **95**: 59-64.
[218] Bebbington A, Parkin I, James PA, Chichester LJ, Kubiak EM Patients' case-notes: look but don't touch. *J Hosp Infect* 2003; **55**: 299-301.
[219] Lemmen SW, Hafner H, Zollman D, Stanzel S, Lutticken R Distribution of multi-resistant Gram-negative versus Gram-positive bacteria in the hospital inanimate environment. *J Hosp Infect* 2004; **56**: 191-7.
[220] Faires MC, Pearl DL, Berke O, Reid-Smith RJ, Weese JS The identification and epidemiology of meticillin-resistant Staphylococcus aureus and Clostridium difficile in patient rooms and the ward environment. *Bmc Infect Dis* 2013; **13**.
[221] Kurashige EJO, Oie S, Furukawa H Contamination of environmental surfaces by methicillin-resistant Staphylococcus aureus (MRSA) in rooms of inpatients with MRSA-positive body sites. *Braz J Microbiol* 2016; **47**: 703-5.
[222] Brooks B, Olm MR, Firek BA *et al.* The developing premature infant gut microbiome is a major factor shaping the microbiome of neonatal intensive care unit rooms. *Microbiome* 2018; **6**.
[223] Afle FCD, Agbankpe AJ, Johnson RC, Houngbegnon O, Houssou SC, Bankole HS Healthcare-associated infections: bacteriological characterization of the hospital surfaces in the University Hospital of Abomey-Calavi/so-ava in South Benin (West Africa). *Bmc Infect Dis* 2019; **19**.
[224] Bhatta DR, Hamal D, Shrestha R *et al.* Bacterial contamination of frequently touched objects in a tertiary care hospital of Pokhara, Nepal: how safe are our hands? *Antimicrob Resist In* 2018; **7**.
[225] Bouchra O, Abdelhakim EL, Nadia B, Btissam A, Wafaa B Environmental surfaces in healthcare setting: a great potential risk of pathogens transmission. *Biomed Res-India* 2017; **28**: 2398-401.

[226] Farias PG, Gama F, Reis D *et al.* Hospital microbial surface colonization revealed during monitoring of Klebsiella spp., Pseudomonas aeruginosa, and non-tuberculous mycobacteria. *Anton Leeuw Int J G* 2017; **110**: 863-76.
[227] Frickmann H, Bachert S, Warnke P, Podbielski A Validated measurements of microbial loads on environmental surfaces in intensive care units before and after disinfecting cleaning. *J Appl Microbiol* 2018; **124**: 874-80.
[228] Matinyi S, Enoch M, Akia D *et al.* Contamination of microbial pathogens and their antimicrobial pattern in operating theatres of peri-urban eastern Uganda: a cross-sectional study. *Bmc Infect Dis* 2018; **18**.
[229] Michael KE, No D, Daniell WE, Seixas NS, Roberts MC Assessment of Environmental Contamination with Pathogenic Bacteria at a Hospital Laundry Facility. *Ann Work Expos Heal* 2017; **61**: 1087-96.
[230] Moghadampour M, Salari-Jazi A, Faghri J High Rate of Carbapenem-Resistant Klebsiella Pneumoniae Detected from Hospital Equipments in Iran. *Acta Microbiol Imm H* 2018; **65**: 529-38.
[231] Phoon HYP, Hussin H, Hussain BM *et al.* Distribution, genetic diversity and antimicrobial resistance of clinically important bacteria from the environment of a tertiary hospital in Malaysia. *J Glob Antimicrob Re* 2018; **14**: 132-40.
[232] Rock C, Anderson M, Lewis S *et al.* Comparison of nylon-flocked swab and cellulose sponge methods for carbapenem-resistant Enterobacteriaceae and gram-negative organism recovery from high-touch surfaces in patient rooms. *Infect Cont Hosp Ep* 2018; **39**: 1257-61.
[233] Shek K, Patidar R, Kohja Z *et al.* Rate of contamination of hospital privacy curtains on a burns and plastic surgery ward: a cross-sectional study. *J Hosp Infect* 2017; **96**: 54-8.
[234] Shek K, Patidar R, Kohja Z *et al.* Rate of contamination of hospital privacy curtains in a burns/plastic ward: A longitudinal study. *Am J Infect Control* 2018; **46**: 1019-21.
[235] Sserwadda I, Lukenge M, Mwambi B, Mboowa G, Walusimbi A, Segujja F Microbial contaminants isolated from items and work surfaces in the post-operative ward at Kawolo general hospital, Uganda. *Bmc Infect Dis* 2018; **18**.
[236] Torkar KG, Ivic S Surveillance of bacterial colonisation on contact surfaces in different medical wards. *Arh Hig Rada Toksiko* 2017; **68**: 116-26.
[237] Widmer FC, Frei R, Romanyuk A, Sutter ST, Widmer AF Overall bioburden by total colony count does not predict the presence of pathogens with high clinical relevance in hospital and community environments. *J Hosp Infect* 2019; **101**: 240-4.
[238] Chen KH, Chen LR, Wang YK Contamination of medical charts: an important source of potential infection in hospitals. *Plos One* 2014; **9**: e78512.
[239] Gbaguidi-Haore H, Talon D, Hocquet D, Bertrand X Hospital environmental contamination with Enterobacteriaceae producing extended-spectrum beta-lactamase. *Am J Infect Control* 2013; **41**: 664-5.
[240] Lu PL, Siu LK, Chen TC *et al.* Methicillin-resistant Staphylococcus aureus and Acinetobacter baumannii on computer interface surfaces of hospital wards and association with clinical isolates. *Bmc Infect Dis* 2009; **9**.
[241] Tajeddin E, Rashidan M, Razaghi M *et al.* The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections. *J Infect Public Heal* 2016; **9**: 13-33.
[242] Tan TY, Tan JSM, Tay H, Chua GH, Ng LSY, Syahidah N Multidrug-resistant organisms in a routine ward environment: differential propensity for

environmental dissemination and implications for infection control. *J Med Microbiol* 2013; **62**: 766-72.

[243] Bures S, Fishbain JT, Uyehara CF, Parker JM, Berg BW Computer keyboards and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit. *Am J Infect Control* 2000; **28**: 465-71.

[244] Carducci A, Verani M, Lombardi R, Casini B, Privitera G Environmental survey to assess viral contamination of air and surfaces in hospital settings. *J Hosp Infect* 2011; **77**: 242-7.

[245] Creamer E, Shore AC, Deasy EC *et al.* Air and surface contamination patterns of meticillin-resistant Staphylococcus aureus on eight acute hospital wards. *J Hosp Infect* 2014; **86**: 201-8.

[246] Dziri R, Klibi N, Lozano C *et al.* High prevalence of Staphylococcus haemolyticus and Staphylococcus saprophyticus in environmental samples of a Tunisian hospital. *Diagn Micr Infec Dis* 2016; **85**: 136-40.

[247] Ford CD, Lopansri BK, Gazdik MA *et al.* Room contamination, patient colonization pressure, and the risk of vancomycin-resistant Enterococcus colonization on a unit dedicated to the treatment of hematologic malignancies and hematopoietic stem cell transplantation. *Am J Infect Control* 2016; **44**: 1110-5.

[248] Gavalda L, Pequeno S, Soriano A, Dominguez MA Environmental contamination by multidrug-resistant microorganisms after daily cleaning. *Am J Infect Control* 2015; **43**: 776-8.

[249] Hartmann B, Benson M, Junger A *et al.* Computer keyboard and mouse as a reservoir of pathogens in an intensive care unit. *J Clin Monit Comput* 2004; **18**: 7-12.

[250] Rohr U, Kaminski A, Wilhelm M, Jurzik L, Gatermann S, Muhr G Colonization of patients and contamination of the patients' environment by MRSA under conditions of single-room isolation. *Int J Hyg Envir Heal* 2009; **212**: 209-15.

[251] Verity P, Wilcox MH, Fawley W, Parnell P Prospective evaluation of environmental contamination by Clostridium difficile in isolation side rooms. *J Hosp Infect* 2001; **49**: 204-9.

[252] Wang YL, Chen WC, Chen YY *et al.* Bacterial contamination on surfaces of public areas in hospitals. *J Hosp Infect* 2010; **74**: 195-6.

[253] Gad GF, El-Domany RA, Zaki S, Ashour HM Characterization of Pseudomonas aeruginosa isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. *J Antimicrob Chemoth* 2007; **60**: 1010-7.

[254] D'Arcy N, Cloutman-Green E, Klein N, Spratt DA Environmental viral contamination in a pediatric hospital outpatient waiting area: Implications for infection control. *Am J Infect Control* 2014; **42**: 856-60.

[255] Ganime AC, Leite JPG, Figueiredo CED *et al.* Dissemination of human adenoviruses and rotavirus species A on fomites of hospital pediatric units. *Am J Infect Control* 2016; **44**: 1411-3.

[256] Silva LKD, Arantes TS, Andrade KR *et al.* High positivity of mimivirus in inanimate surfaces of a hospital respiratory-isolation facility, Brazil. *J Clin Virol* 2015; **66**: 62-5.

[257] Dubberke ER, Reske KA, Noble-Wang J *et al.* Prevalence of Clostridium difficile environmental contamination and strain variability in multiple health care facilities. *Am J Infect Control* 2007; **35**: 315-8.

[258] Shams AM, Rose LJ, Edwards JR *et al.* Assessment of the Overall and Multidrug-Resistant Organism Bioburden on Environmental Surfaces in Healthcare Facilities. *Infect Cont Hosp Ep* 2016; **37**: 1426-32.

[259] Anderson K, Sallis, P., Uyanik, S. *Handbook of Water and Wastewater Microbiology*: Academic Press 2003.
[260] Griffith C Surface Sampling and the Detection of Contamination. *Handbook of Hygiene Control in the Food Industry* 2016; doi 10.1016/B978-0-08-100155-4.00044-3: 673-96.
[261] Bonnet M, Lagier JC, Raoult D, Khelaifia S Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New Microbes New Infect* 2020; **34**: 100622.
[262] Ibfelt T, Foged C, Andersen LP Validation of dipslides as a tool for environmental sampling in a real-life hospital setting. *Eur J Clin Microbiol* 2014; **33**: 809-13.
[263] Moore G, Griffith C Problems associated with traditional hygiene swabbing: the need for in-house standardization. *J Appl Microbiol* 2007; **103**: 1090-103.
[264] Dey BP, Engley, F.B. Methodology for recovery of chemically treated Staphylococcus aureus with neutralizing medium. *Appl Environ Microb* 1983; **45**: 1533-7.
[265] Dey BP, Engley FB, Jr. Comparison of Dey and Engley (D/E) neutralizing medium to Letheen medium and standard methods medium for recovery of Staphylococcus aureus from sanitized surfaces. *J Ind Microbiol* 1995; **14**: 21-5.
[266] Griffith C. Handbook of Hygiene Control in the Food Industry. In: Lelieveld HLMM, M.A. Holah, J., Ed. *Improving surface sampling and detection of contamination*: Woodhead Publishing 2005; 588-618.
[267] Pinto F, Hiom S, Girdlestone S, Maillard JY Evaluation of the effectiveness of commercially available contact plates for monitoring microbial environments. *Lett Appl Microbiol* 2009; **48**: 379-82.
[268] Rabuza U, Sostar-Turk S, Fijan S Efficiency of four sampling methods used to detect two common nosocomial pathogens on textiles. *Text Res J* 2012; **82**: 2099-105.
[269] Obee P, Griffith CJ, Cooper RA, Bennion NE An evaluation of different methods for the recovery of meticillin-resistant Staphylococcus aureus from environmental surfaces. *J Hosp Infect* 2007; **65**: 35-41.
[270] Claro T, Galvin S, Cahill O, Fitzgerald-Hughes D, Daniels S, Humphreys H What Is the Best Method? Recovery of Methicillin-Resistant Staphylococcus aureus and Extended-Spectrum beta-Lactamase-Producing Escherichia coli from Inanimate Hospital Surfaces. *Infect Cont Hosp Ep* 2014; **35**: 869-71.
[271] Hogan PG, Burnham CA, Singh LN *et al.* Evaluation of Environmental Sampling Methods for Detection of Staphylococcus aureus on Fomites. *Ann Public Health Res* 2015; **2**.
[272] Nyachuba DG, Donnelly CW Comparison of 3M (TM) Petrifilm (TM) environmental Listeria plates against standard enrichment methods for the detection of Listeria monocytogenes of epidemiological significance from environmental surfaces. *J Food Sci* 2007; **72**: M346-M54.
[273] Verran J, Redfern J, Smith LA, Whitehead KA A critical evaluation of sampling methods used for assessing microorganisms on surfaces. *Food Bioprod Process* 2010; **88**: 335-40.
[274] Hedin G, Rynback J, Lore B New technique to take samples from environmental surfaces using flocked nylon swabs. *J Hosp Infect* 2010; **75**: 314-7.
[275] Dalmaso G, Bini M, Paroni R, Ferrari M Qualification of high-recovery, flocked swabs as compared to traditional rayon swabs for microbiological environmental monitoring of surfaces. *PDA J Pharm Sci Technol* 2008; **62**: 191-9.

[276] Park GW, Lee D, Treffiletti A, Hrsak M, Shugart J, Vinje J Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces. *Appl Environ Microbiol* 2015; **81**: 5987-92.

[277] Otter JA, Havill NL, Adams NMT, Cooper T, Tauman A, Boyce JM Environmental sampling for Clostridium difficile: Swabs or sponges? *Am J Infect Control* 2009; **37**: 517-8.

[278] Grainger RJ, Stevens NT, Humphreys H Approaches to the detection of Clostridioides difficile in the healthcare environment. *J Hosp Infect* 2019; **103**: 375-81.

[279] Brown KA, MacDougall LK, Valenta K *et al.* Increased environmental sample area and recovery of Clostridium difficile spores from hospital surfaces by quantitative PCR and enrichment culture. *Infect Control Hosp Epidemiol* 2018; **39**: 917-23.

[280] Estill CF, Baron PA, Beard JK *et al.* Recovery efficiency and limit of detection of aerosolized Bacillus anthracis Sterne from environmental surface samples. *Appl Environ Microbiol* 2009; **75**: 4297-306.

[281] Ali S, Muzslay M, Wilson P A Novel Quantitative Sampling Technique for Detection and Monitoring of Clostridium difficile Contamination in the Clinical Environment. *J Clin Microbiol* 2015; **53**: 2570-4.

[282] Alothman A, Jelani A, Althaqafi A, Rich M, Williams E Contamination of patient hospital charts by bacteria. *J Hosp Infect* 2003; **55**: 304-5.

[283] Downey AS, Da Silva SM, Olson ND, Filliben JJ, Morrow JB Impact of Processing Method on Recovery of Bacteria from Wipes Used in Biological Surface Sampling. *Appl Environ Microb* 2012; **78**: 5872-81.

[284] Chaibenjawong P, Foster SJ Desiccation tolerance in Staphylococcus aureus. *Arch Microbiol* 2011; **193**: 125-35.

[285] Potts M, Slaughter SM, Hunneke FU, Garst JF, Helm RF Desiccation tolerance of prokaryotes: application of principles to human cells. *Integr Comp Biol* 2005; **45**: 800-9.

[286] Hirai Y Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. *J Hosp Infect* 1991; **19**: 191-200.

[287] Roelofsen E, van Leeuwen M, Meijer-Severs GJ, Wilkinson MH, Degener JE Evaluation of the effects of storage in two different swab fabrics and under three different transport conditions on recovery of aerobic and anaerobic bacteria. *J Clin Microbiol* 1999; **37**: 3041-3.

[288] Keeratipibul S, Laovittayanurak, T., Pornruangsarp, O., Chaturongkasumrit, Y., Takahashi, H., Techaruvichit, P. Effect of swabbing techniques on the efficiency of bacterial recovery from food contact surfaces. *Food Control* 2017; **77**: 139-44.

[289] Kelly S, Hardwick R, Wong J, Rao GG Laboratory evaluation of selective mannitol broth for MRSA screening. *J Hosp Infect* 2004; **58**: 239-40.

[290] Paksanont S, Sintiprungrat K, Yimthin T, Pumirat P, Peacock SJ, Chantratita N Effect of temperature on Burkholderia pseudomallei growth, proteomic changes, motility and resistance to stress environments. *Sci Rep* 2018; **8**: 9167.

[291] Artasensi A, Mazzotta S, Fumagalli L Back to Basics: Choosing the Appropriate Surface Disinfectant. *Antibiotics (Basel)* 2021; **10**.

[292] Malanovic N, Lohner K Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides. *Biochim Biophys Acta* 2016; **1858**: 936-46.

[293] Kramer A, Schwebke I, Kampf G How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *Bmc Infect Dis* 2006; **6**.

[294] Lahou E, Uyttendaele M Evaluation of Three Swabbing Devices for Detection of Listeria monocytogenes on Different Types of Food Contact Surfaces. *Int J Env Res Pub He* 2014; **11**: 804-14.
[295] Brummer B Influence of Possible Disinfectant Transfer on Staphylococcus-Aureus Plate Counts after Agar Contact Sampling. *Appl Environ Microb* 1976; **32**: 80-4.
[296] Boal AK, Patsalis, F.I. Use of Sodium Thiosulfate to Quench Hypochlorite Solutions Prior to Chlorate Analysis. *Journal of American Water Works Association* 2017; **109**: 410-5.
[297] Lutz JK, Crawford J, Hoet AE, Wilkins JR, Lee J Comparative performance of contact plates, electrostatic wipes, swabs and a novel sampling device for the detection of Staphylococcus aureus on environmental surfaces. *J Appl Microbiol* 2013; **115**: 171-8.
[298] Flint SH, Brooks, J.D, Bremer, P.J Properties of the stainless steel substrate, influencing the adhesion of thermo-resistant streptococci. *Journal of Food Engineering* 2000; **43**: 235-42.
[299] Hadla M, Halabi, M. *Comprehensive Analytical Chemistry*: Elsevier 2018.
[300] Schinner S, Engelhardt, F., Preusse, M., Thoming, J.G., Tomasch, J., Haussler, S Genetic determinants of Pseudomonas aeruginosa fitness during biofilm growth. *Biofilm* 2020; **2**.
[301] Musken M, Di Fiore S, Dotsch A, Fischer R, Haussler S Genetic determinants of Pseudomonas aeruginosa biofilm establishment. *Microbiology* 2010; **156**: 431-41.
[302] Tuson HH, Weibel DB Bacteria-surface interactions. *Soft Matter* 2013; **9**: 4368-80.
[303] Chew SCY, L. Encyclopedia of Food and Health. In: Caballero B, Finglas, P.M., Toldra, F., Ed. *Biofilms*: Academic Press 2016; 407-15.
[304] Ceyhan N, Ozdemir G Extracellular polysaccharides produced by cooling water tower biofilm bacteria and their possible degradation. *Biofouling* 2008; **24**: 129-35.
[305] Berne C, Ducret A, Hardy GG, Brun YV Adhesins Involved in Attachment to Abiotic Surfaces by Gram-Negative Bacteria. *Microbiol Spectr* 2015; **3**.
[306] Jacobsen T, Bardiaux B, Francetic O, Izadi-Pruneyre N, Nilges M Structure and function of minor pilins of type IV pili. *Med Microbiol Immunol* 2020; **209**: 301-8.
[307] Arunasri K, Mohan, S.V. *Microbial Electrochemical Technology* Elsevier 2019.
[308] Aykent F, Yondem I, Ozyesil AG, Gunal SK, Avunduk MC, Ozkan S Effect of different finishing techniques for restorative materials on surface roughness and bacterial adhesion. *The Journal of prosthetic dentistry* 2010; **103**: 221-7.
[309] Hood SK, Zottola EA Adherence to stainless steel by foodborne microorganisms during growth in model food systems. *Int J Food Microbiol* 1997; **37**: 145-53.
[310] Lewis JA Colloidal Processing of Ceramics. *Journal of the American Ceramic Society* 2000; **83**: 2341-59.
[311] Motz VA, Young, L.M., Motz, M.E., Young, S.C A Sticking Point in Assessing Bacterial Contamination: Adhesive Characters of Bacterial Specializations, Swab Features, and Fomite Surface Properties Skew Colony Counts. *J Pure Appl Microbio* 2019; **13**: 2533-44.
[312] Gottenbos B, Grijpma DW, van der Mei HC, Feijen J, Busscher HJ Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. *The Journal of antimicrobial chemotherapy* 2001; **48**: 7-13.

[313] Dickson JS, Koohmaraie M Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Appl Environ Microbiol* 1989; **55**: 832-6.
[314] Jucker BA, Harms H, Zehnder AJ Adhesion of the positively charged bacterium Stenotrophomonas (Xanthomonas) maltophilia 70401 to glass and Teflon. *J Bacteriol* 1996; **178**: 5472-9.
[315] Hayashi H, Seiki H, Tsuneda S, Hirata A, Sasaki H Influence of growth phase on bacterial cell electrokinetic characteristics examined by soft particle electrophoresis theory. *J Colloid Interface Sci* 2003; **264**: 565-8.
[316] Midelet G, Carpentier B Transfer of microorganisms, including Listeria monocytogenes, from various materials to beef. *Appl Environ Microbiol* 2002; **68**: 4015-24.
[317] Rawlinson S, Cloutman-Green, E., Asadi, F., Ciric, L Surface sampling within a pediatric ward—how multiple factors affect cleaning efficacy. *Am J Infect Control* 2020; **48**: 740-5.
[318] Hedges AJ Estimating the precision of serial dilutions and viable bacterial counts. *Int J Food Microbiol* 2002; **76**: 207-14.
[319] Peleg AY, Hooper DC Hospital-acquired infections due to gram-negative bacteria. *N Engl J Med* 2010; **362**: 1804-13.
[320] Foster T. Staphylococcus. In: th, Baron S, Eds. *Medical Microbiology* Galveston (TX) 1996.
[321] Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME Staphylococcus aureus biofilms: properties, regulation, and roles in human disease. *Virulence* 2011; **2**: 445-59.
[322] Alcantar-Curiel MD, Blackburn D, Saldana Z *et al.* Multi-functional analysis of Klebsiella pneumoniae fimbrial types in adherence and biofilm formation. *Virulence* 2013; **4**: 129-38.
[323] Ghasemian A, Mobarez, A.M., Peerayeh, SN., Bezmin Abadi, A.T The association of surface adhesin genes and the biofilm formation among Klebsiella oxytoca clinical isolates. *New Microbes and New Infections* 2019; **27**: 36-9.
[324] Lebreton F, Willems RJL, Gilmore MS. Enterococcus Diversity, Origins in Nature, and Gut Colonization. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, Eds. *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection* Boston 2014.
[325] Stuart CH, Schwartz SA, Beeson TJ, Owatz CB Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. *J Endod* 2006; **32**: 93-8.
[326] Iglewski BH. Pseudomonas. In: th, Baron S, Eds. *Medical Microbiology* Galveston (TX) 1996.
[327] Rasamiravaka T, Labtani Q, Duez P, El Jaziri M The formation of biofilms by Pseudomonas aeruginosa: a review of the natural and synthetic compounds interfering with control mechanisms. *Biomed Res Int* 2015; **2015**: 759348.
[328] Kjelleberg S, Humphrey BA, Marshall KC Initial phases of starvation and activity of bacteria at surfaces. *Appl Environ Microbiol* 1983; **46**: 978-84.
[329] Nadell CD, Drescher K, Foster KR Spatial structure, cooperation and competition in biofilms. *Nat Rev Microbiol* 2016; **14**: 589-600.
[330] CDC. Guideline for Disinfection and Sterilization in Healthcare Facilities (2008). In: Control I, Ed.: Centers for Disease Control and Prevention 2008.
[331] Donskey CJ Does improving surface cleaning and disinfection reduce health care-associated infections? *Am J Infect Control* 2013; **41**: S12-9.
[332] WHO. Decontamination and Reprocessing of Medical Devices for Health-care Facilities. In: World Health Organization PAHO, Ed.: 2016.

[333] Peters A, Otter, J., Moldovan, A., Parneix, P., Voss, A., Pittet, D. Keeping hospitals clean and safe without breaking the bank; summary of the Healthcare Cleaning Forum 2018. *Antimicrob Resist In* 2018; **7**.
[334] Kim CY, Ryu, G.J., Park, H.Y., Ryu, K. Resistance of Staphylococcus aureus on food contact surfaces with different surface characteristics to chemical sanitizers. *J Food Saf* 2017; **37**: e12354.
[335] Bancalari E, Martelli, F., Bernini, V., Neviani, E., Gatti, M. Bacteriostatic or bactericidal? Impedometric measurements to test the antimicrobial activity of Arthrospira platensis extract. *Food Control* 2020; **118**.
[336] Dancer SJ, Kramer A Four steps to clean hospitals: LOOK, PLAN, CLEAN and DRY. *J Hosp Infect* 2019; **103**: e1-e8.
[337] NHS. The national specifications for cleanliness in the NHS: a framework for setting and measuring performance outcomes. In: Agency NPS, Ed.: 2007.
[338] Pratt RJ, Pellowe CM, Wilson JA *et al.* epic2: National evidence-based guidelines for preventing healthcare-associated infections in NHS hospitals in England. *J Hosp Infect* 2007; **65 Suppl 1**: S1-64.
[339] Gallandat K, Kolus RC, Julian TR, Lantagne DS A systematic review of chlorine-based surface disinfection efficacy to inform recommendations for low-resource outbreak settings. *Am J Infect Control* 2021; **49**: 90-103.
[340] Russell AD Bacterial resistance to disinfectants: present knowledge and future problems. *J Hosp Infect* 1999; **43 Suppl**: S57-68.
[341] Cheng KC, Khoo ZS, Lo NW, Tan WJ, Chemmangattuvalappil NG Design and performance optimisation of detergent product containing binary mixture of anionic-nonionic surfactants. *Heliyon* 2020; **6**: e03861.
[342] Williams JJ. Handbook for Cleaning/Decontamination of Surfaces. In: Johansson K, Somasundaran, P., Ed. *Handbook for Cleaning/Decontamination of Surfaces*: Elsevier Science 2007; 103-23.
[343] Chan HTW, P.; Sheorey, H.; Cocks, J.; Waters, M. J. Evaluation of the biological efficacy of hydrogen peroxide vapour decontamination in wards of an Australian hospital. *J Hosp Infect* 2011; **79**: 125-8.
[344] Shaban SM, Kang, J., Kim, D-H Surfactants: Recent advances and their applications. *Composites Communications* 2020; **22**.
[345] Ramm L, Siani H, Wesgate R, Maillard JY Pathogen transfer and high variability in pathogen removal by detergent wipes. *Am J Infect Control* 2015; **43**: 724-8.
[346] Rutala WA, Gergen MF, Weber DJ Microbiologic evaluation of microfiber mops for surface disinfection. *Am J Infect Control* 2007; **35**: 569-73.
[347] Siani H, Wesgate R, Maillard J-Y Impact of antimicrobial wipes compared with hypochlorite solution on environmental surface contamination in a health care setting: A double-crossover study. *Am J Infect Control* 2018; doi https://doi.org/10.1016/j.ajic.2018.03.020.
[348] Ungurs M, Wand M, Vassey M *et al.* The effectiveness of sodium dichloroisocyanurate treatments against Clostridium difficile spores contaminating stainless steel. *Am J Infect Control* 2011; **39**: 199-205.
[349] Vermaas JFH, C. J.; Steyn, H. J. H.; Schall, R. THE EFFICACY OF ANOLYTE AS AN ENVIRONMENTALLY FRIENDLY DISINFECTANT ON ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS CONTAMINATED COTTON, POLYESTER/COTTON AND POLYESTER. *J Fam Ecol Consum Soc* 2015; **43**: 26-32.
[350] Williams GJ, Denyer SP, Hosein IK, Hill DW, Maillard JY Limitations of the Efficacy of Surface Disinfection in the Healthcare Setting. *Infect Cont Hosp Ep* 2009; **30**: 570-3.
[351] Aarnisalo KS, S.; Miettinen, H.; Suihko, M. L.; Wirtanen, G.; Autio, T.; Lunden, J.; Korkeala, H.; Sjoberg, A. M. Bactericidal efficiencies of

commercial disinfectants against Listeria monocytogenes on surfaces. *J Food Saf* 2000; **20**: 237-50.
[352] Best M, Sattar SA, Springthorpe VS, Kennedy ME Efficacies of selected disinfectants against Mycobacterium tuberculosis. *J Clin Microbiol* 1990; **28**: 2234-9.
[353] Chiu SS, Brenton; Petric, Martin; McIntyre, Lorraine; Gamage, Bruce; Isaac-Renton, Judith Efficacy of common disinfectant/cleaning agents in inactivating murine norovirus and feline calicivirus as surrogate viruses for human norovirus. *Am J Infect Control* 2015; **43**: 1208-12.
[354] Guan JWC, M.; Brooks, B. W.; Rohonczy, L. Influence of temperature and organic load on chemical disinfection of Geobacillus steareothermophilus spores, a surrogate for Bacillus anthracis. *Can J Vet Res-Rev Can Rech Vet* 2013; **77**: 100-4.
[355] Lambert RJ, Johnston MD The effect of interfering substances on the disinfection process: a mathematical model. *J Appl Microbiol* 2001; **91**: 548-55.
[356] Tulis JJ. *Industrial Sterilization,* Durham: Duke University Press 1972.
[357] Bock LJ Bacterial biocide resistance: a new scourge of the infectious disease world? *Arch Dis Child* 2019; **104**: 1029-33.
[358] Brown SA, Merritt K, Woods TO, Busick DN Effects on instruments of the World Health Organization--recommended protocols for decontamination after possible exposure to transmissible spongiform encephalopathy-contaminated tissue. *J Biomed Mater Res B Appl Biomater* 2005; **72**: 186-90.
[359] Crawford M How clean is clean?: chemistry can damage medical equipment in the quest to meet stringent guidelines. *Biomed Instrum Technol* 2014; **48**: 260-3.
[360] Dettenkofer M, Block C Hospital disinfection: efficacy and safety issues. *Curr Opin Infect Dis* 2005; **18**: 320-5.
[361] Harbarth S, Tuan Soh S, Horner C, Wilcox MH Is reduced susceptibility to disinfectants and antiseptics a risk in healthcare settings? A point/counterpoint review. *J Hosp Infect* 2014; **87**: 194-202.
[362] Boyce JM A review of wipes used to disinfect hard surfaces in health care facilities. *Am J Infect Control* 2021; **49**.
[363] Song X, Vossebein L, Zille A Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: a review. *Antimicrob Resist Infect Control* 2019; **8**: 139.
[364] Russo MW How to Review a Meta-analysis. *Gastroenterol Hepatol (N Y)* 2007; **3**: 637-42.
[365] Donaghy JA, Jagadeesan B, Goodburn K *et al.* Relationship of Sanitizers, Disinfectants, and Cleaning Agents with Antimicrobial Resistance. *J Food Prot* 2019; **82**: 889-902.
[366] Kampf G Adaptive microbial response to low-level benzalkonium chloride exposure. *J Hosp Infect* 2018; **100**: e1-e22.
[367] Braoudaki M, Hilton AC Adaptive resistance to biocides in Salmonella enterica and Escherichia coli O157 and cross-resistance to antimicrobial agents. *J Clin Microbiol* 2004; **42**: 73-8.
[368] Sifuentes LY, Gerba CP, Weart I, Engelbrecht K, Koenig DW Microbial contamination of hospital reusable cleaning towels. *Am J Infect Control* 2013; **41**: 912-5.
[369] Werry C, Lawrence JM, Sanderson PJ Contamination of detergent cleaning solutions during hospital cleaning. *J Hosp Infect* 1988; **11**: 44-9.
[370] Boyce JM, Havill NL In-use contamination of a hospital-grade disinfectant. *Am J Infect Control* 2022; doi 10.1016/j.ajic.2022.03.008.

[371] Takashima M, Shirai F, Sageshima M, Ikeda N, Okamoto Y, Dohi Y Distinctive bacteria-binding property of cloth materials. *Am J Infect Control* 2004; **32**: 27-30.
[372] Varshney S, Sain, A., Gupta, D., Sharma, S. Factors Affecting Bacterial Adhesion on Selected Textile Fibres. *Indian Journal of Microbiology* 2020; doi 10.1007/s12088-020-00903-5.
[373] Obee P, Griffith, C., Greten, R.Z. Efficacy of disinfectants and detergents for cleaning hospital environmental surfaces as part of documented cleaning protocols. *Am J Infect Control* 2005; **33**: e38-e9.
[374] Gonzalez EA, Nandy P, Lucas AD, Hitchins VM Ability of cleaning-disinfecting wipes to remove bacteria from medical device surfaces. *Am J Infect Control* 2015; **43**: 1331-5.
[375] Gold KM, Hitchins VM Cleaning assessment of disinfectant cleaning wipes on an external surface of a medical device contaminated with artificial blood or Streptococcus pneumoniae. *Am J Infect Control* 2013; **41**: 901-7.
[376] Gan T, Xu H, Wu J *et al.* Sequential enhanced cleaning eliminates multidrug-resistant organisms in general intensive care unit of a traditional Chinese medicine hospital. *J Crit Care* 2017; **41**: 216-21.
[377] Ni KW, Chen BB, Jin H, Kong QX, Ni XP, Xu H Knowledge, attitudes, and practices regarding environmental cleaning among environmental service workers in Chinese hospitals. *Am J Infect Control* 2017; **45**: 1043-5.
[378] Mitchell BG, White N, Farrington A *et al.* Changes in knowledge and attitudes of hospital environmental services staff: The Researching Effective Approaches to Cleaning in Hospitals (REACH) study. *Am J Infect Control* 2018; doi 10.1016/j.ajic.2018.02.003.
[379] Carling PC, Parry MF, Bruno-Murtha LA, Dick B Improving environmental hygiene in 27 intensive care units to decrease multidrug-resistant bacterial transmission*. *Critical Care Medicine* 2010; **38**: 1054-9.
[380] Dancer SJ How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals. *Journal of Hospital Infection* 2004; **56**: 10-5.
[381] Dancer SJ, Coyne M, Speekenbrink A, Samavedam S, Kennedy J, Wallace PGM MRSA acquisition in an intensive care unit. *American Journal of Infection Control* 2006; **34**: 10-7.
[382] Bhalla AMD, Pultz NJBS, Gries DMMD *et al.* Acquisition of Nosocomial Pathogens on Hands After Contact With Environmental Surfaces Near Hospitalized Patients • *Infection Control and Hospital Epidemiology* 2004; **25**: 164-7.
[383] Al-Hamad A, Maxwell S How clean is clean? Proposed methods for hospital cleaning assessment. *J Hosp Infect* 2008; **70**: 328-34.
[384] Posfay-Barbe KM, Zerr DM, Pittet D Infection control in paediatrics. *Lancet Infect Dis* 2008; **8**: 19-31.
[385] Dancer SJ Mopping up hospital infection. *J Hosp Infect* 1999; **43**: 85-100.
[386] Hardy KJ, Gossain S, Henderson N *et al.* Rapid recontamination with MRSA of the environment of an intensive care unit after decontamination with hydrogen peroxide vapour. *J Hosp Infect* 2007; **66**: 360-8.
[387] White LF, Dancer SJ, Robertson C A microbiological evaluation of hospital cleaning methods. *Int J Environ Health Res* 2007; **17**: 285-95.
[388] Cohen B, Hyman S, Rosenberg L, Larson E Frequency of patient contact with health care personnel and visitors: implications for infection prevention. *Jt Comm J Qual Patient Saf* 2012; **38**: 560-5.
[389] Sifuentes LY, Fankem SL, Reynolds K, Tamimi AH, Gerba CP, Koenig D Use of ATP Readings to Predict a Successful Hygiene Intervention in the

Workplace to Reduce the Spread of Viruses on Fomites. *Food Environ Virol* 2017; **9**: 14-9.

[390] Watson F, Chewins, J., Wilks, S., Keevil, B Underestimating the Levels of Microbiological Contamination on Environmental Surfaces Within NHS Hospitals. *Am J Infect Control* 2020; **48**: S22.

[391] Dancer SJ Hospital cleaning in the 21st century. *Eur J Clin Microbiol Infect Dis* 2011; **30**: 1473-81.

[392] Manian FA, Griesnauer S, Bryant A Implementation of hospital-wide enhanced terminal cleaning of targeted patient rooms and its impact on endemic Clostridium difficile infection rates. *Am J Infect Control* 2013; **41**: 537-41.

[393] Wilson AP, Smyth D, Moore G *et al.* The impact of enhanced cleaning within the intensive care unit on contamination of the near-patient environment with hospital pathogens: a randomized crossover study in critical care units in two hospitals. *Crit Care Med* 2011; **39**: 651-8.

[394] Schneider J, Moromisato D, Zemetra B *et al.* Hand hygiene adherence is influenced by the behavior of role models. *Pediatr Crit Care Med* 2009; **10**: 360-3.

[395] Srigley JA, Cho, S.M., O'Neill, C., Bialachowski, A., Ali, R.A., Lee, C., Mertz, D. Hand hygiene knowledge, attitudes, and practices among hospital inpatients: A descriptive study. *Am J Infect Control* 2020; **48**: 507-10.

[396] Fries J, Segre AM, Thomas G, Herman T, Ellingson K, Polgreen PM Monitoring hand hygiene via human observers: how should we be sampling? *Infect Control Hosp Epidemiol* 2012; **33**: 689-95.

[397] Freeman MC, Stocks ME, Cumming O *et al.* Hygiene and health: systematic review of handwashing practices worldwide and update of health effects. *Trop Med Int Health* 2014; **19**: 906-16.

[398] Huttunen R, Syrjänen, J. Healthcare workers as vectors of infectious diseases. *Eur J Clin Microbiol* 2014; **33**: 1477-88.

[399] Lankford MG, Zembower TR, Trick WE, Hacek DM, Noskin GA, Peterson LR Influence of role models and hospital design on hand hygiene of healthcare workers. *Emerg Infect Dis* 2003; **9**: 217-23.

[400] Mitchell BG, Farrington A, Allen M *et al.* Variation in hospital cleaning practice and process in Australian hospitals: A structured mapping exercise. *Infect Dis Health* 2017; **22**: 195-202.

[401] Ivers N, Jamtvedt G, Flottorp S *et al.* Audit and feedback: effects on professional practice and healthcare outcomes. *Cochrane Database Syst Rev* 2012; doi 10.1002/14651858.CD000259.pub3: CD000259.

[402] Charrier L, Allochis MC, Cavallo MR, Gregori D, Cavallo F, Zotti CM Integrated audit as a means to implement unit protocols: a randomized and controlled study. *J Eval Clin Pract* 2008; **14**: 847-53.

[403] WHO. WHO Guidelines on Hand Hygiene in Health Care. 2009.

[404] Wilson J Rethinking the use of audit to drive improvement. *J Infect Prev* 2018; **19**: 3-4.

[405] Boyce JM Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. *Antimicrob Resist Infect Control* 2016; **5**: 10.

[406] Bergen LKM, M.; Hog, M.; Rubenhagen, B.; Andersen, L. P. Spread of bacteria on surfaces when cleaning with microfibre cloths. *J Hosp Infect* 2009; **71**: 132-7.

[407] Sattar SA, Maillard JY The crucial role of wiping in decontamination of high-touch environmental surfaces: review of current status and directions for the future. *Am J Infect Control* 2013; **41**: S97-104.

[408] West AM, Nkemngong CA, Voorn MG *et al.* Surface area wiped, product type, and target strain impact bactericidal efficacy of ready-to-use disinfectant Towelettes. *Antimicrob Resist Infect Control* 2018; **7**: 122.
[409] Sax H, Allegranzi B, Uckay I, Larson E, Boyce J, Pittet D 'My five moments for hand hygiene': a user-centred design approach to understand, train, monitor and report hand hygiene. *J Hosp Infect* 2007; **67**: 9-21.
[410] Bergen LK, Meyer M, Hog M, Rubenhagen B, Andersen LP Spread of bacteria on surfaces when cleaning with microfibre cloths. *J Hosp Infect* 2009; **71**: 132-7.
[411] Ganime AC, Carvalho-Costa FA, Mendonca MC *et al.* Group A rotavirus detection on environmental surfaces in a hospital intensive care unit. *Am J Infect Control* 2012; **40**: 544-7.
[412] Rocha IV, Xavier DE, Almeida KRH, Oliveira SR, Leal NC Multidrug-resistant Acinetobacter baumannii clones persist on hospital inanimate surfaces. *Braz J Infect Dis* 2018; **22**: 438-41.
[413] Carling PC, Von Beheren S, Kim P, Woods C, Healthcare Environmental Hygiene Study G Intensive care unit environmental cleaning: an evaluation in sixteen hospitals using a novel assessment tool. *J Hosp Infect* 2008; **68**: 39-44.
[414] Pierce J, Apisarnthanarak A, Schellack N *et al.* Global Antimicrobial Stewardship with a Focus on Low- and Middle-Income Countries. *Int J Infect Dis* 2020; doi 10.1016/j.ijid.2020.05.126.
[415] Cross S, Gon G, Morrison E *et al.* An invisible workforce: the neglected role of cleaners in patient safety on maternity units. *Glob Health Action* 2019; **12**: 1480085.
[416] Ramphal L, Suzuki S, McCracken IM, Addai A Improving hospital staff compliance with environmental cleaning behavior. *Proc (Bayl Univ Med Cent)* 2014; **27**: 88-91.
[417] Khelifa M, Masse D, Blanc S, Drucker M Evaluation of the minimal replication time of Cauliflower mosaic virus in different hosts. *Virology* 2010; **396**: 238-45.
[418] Franck A, Guilley H, Jonard G, Richards K, Hirth L Nucleotide sequence of cauliflower mosaic virus DNA. *Cell* 1980; **21**: 285-94.
[419] Jiang X, Dai X, Goldblatt S *et al.* Pathogen transmission in child care settings studied by using a cauliflower virus DNA as a surrogate marker. *J Infect Dis* 1998; **177**: 881-8.
[420] Oelberg DG, Joyner SE, Jiang X, Laborde D, Islam MP, Pickering LK Detection of pathogen transmission in neonatal nurseries using DNA markers as surrogate indicators. *Pediatrics* 2000; **105**: 311-5.
[421] Saiki RK, Scharf S, Faloona F *et al.* Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985; **230**: 1350-4.
[422] Saiki RK, Gelfand DH, Stoffel S *et al.* Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; **239**: 487-91.
[423] Mouajou V, Adams K, DeLisle G, Quach C Hand hygiene compliance in the prevention of hospital-acquired infections: a systematic review. *J Hosp Infect* 2022; **119**: 33-48.
[424] Steinmann J Surrogate viruses for testing virucidal efficacy of chemical disinfectants. *J Hosp Infect* 2004; **56 Suppl 2**: S49-54.
[425] Alhmidi H, John A, Mana TC *et al.* Evaluation of Viral Surrogate Markers for Study of Pathogen Dissemination During Simulations of Patient Care. *Open Forum Infect Dis* 2017; **4**: ofx128.

[426] Hall AJ Noroviruses: the perfect human pathogens? *J Infect Dis* 2012; **205**: 1622-4.
[427] Schmid-Hempel P, Frank SA Pathogenesis, virulence, and infective dose. *PLoS Pathog* 2007; **3**: 1372-3.
[428] Greene C, Vadlamudi G, Eisenberg M, Foxman B, Koopman J, Xi C Fomite-fingerpad transfer efficiency (pick-up and deposit) of Acinetobacter baumannii-with and without a latex glove. *Am J Infect Control* 2015; **43**: 928-34.
[429] Legg SJ, Khela N, Madie P, Fenwick SG, Quynh V, Hedderley DI A comparison of bacterial adherence to bare hands and gloves following simulated contamination from a beef carcass. *Int J Food Microbiol* 1999; **53**: 69-74.
[430] Paz V, Paniagua, M., Santillan, A., Alaniz, M., D'Agostino, L., Orellana, R., Rodriguez, C. Hospital environment hygiene nurse: a key player to reduce healthcare associated infections by multi-resistant organisms. *Infection Prevention in Practice* 2020; **2**.
[431] Stiller A, Salm F, Bischoff P, Gastmeier P Relationship between hospital ward design and healthcare-associated infection rates: a systematic review and meta-analysis. *Antimicrob Resist Infect Control* 2016; **5**: 51.
[432] Birnbach DJ, Nevo I, Scheinman SR, Fitzpatrick M, Shekhter I, Lombard JL Patient safety begins with proper planning: a quantitative method to improve hospital design. *Qual Saf Health Care* 2010; **19**: 462-5.
[433] Ellison J, Southern D, Holton D *et al.* Hospital ward design and prevention of hospital-acquired infections: A prospective clinical trial. *Can J Infect Dis Med Microbiol* 2014; **25**: 265-70.
[434] Thompson DR, Hamilton DK, Cadenhead CD *et al.* Guidelines for intensive care unit design. *Crit Care Med* 2012; **40**: 1586-600.
[435] Barraud O, Casellas M, Dagot C, Ploy MC An antibiotic-resistant class 3 integron in an Enterobacter cloacae isolate from hospital effluent. *Clin Microbiol Infect* 2013; **19**: E306-8.
[436] Mitchell BG, Williams A, Wong Z, O'Connor J Assessing a temporary isolation room from an infection control perspective: A discussion paper. *Infect Dis Health* 2017; **22**: 129-35.
[437] Bhattacharyya S, Dey K, Paul AR, Biswas R A novel CFD analysis to minimize the spread of COVID-19 virus in hospital isolation room. *Chaos Solitons Fractals* 2020; **139**: 110294.
[438] Tsui BCH, Pan S Are aerosol-generating procedures safer in an airborne infection isolation room or operating room? *Br J Anaesth* 2020; doi 10.1016/j.bja.2020.09.011.
[439] Barlow GD, Knight J, McKay I *et al.* An audit of the use of side- and isolation room facilities in a UK teaching hospital. *J Hosp Infect* 2006; **62**: 110-2.
[440] Kundrapu S, Sunkesula V, Jury LA, Sitzlar BM, Donskey CJ Daily disinfection of high-touch surfaces in isolation rooms to reduce contamination of healthcare workers' hands. *Infect Control Hosp Epidemiol* 2012; **33**: 1039-42.
[441] Prince AM, Andrus L PCR: how to kill unwanted DNA. *Biotechniques* 1992; **12**: 358-60.
[442] Dancer SJ Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination. *Clin Microbiol Rev* 2014; **27**: 665-90.
[443] Velasco C, Rodríguez-Baño J, García L *et al.* Eradication of an extensive outbreak in a neonatal unit caused by two sequential Klebsiella pneumoniae clones harbouring related plasmids encoding an extended-spectrum β-lactamase. *Journal of Hospital Infection* 2009; **73**: 157-63.

[444] Cheng VC, Chau PH, Lee WM *et al.* Hand-touch contact assessment of high-touch and mutual-touch surfaces among healthcare workers, patients, and visitors. *J Hosp Infect* 2015; **90**: 220-5.
[445] Huslage K, Rutala WA, Sickbert-Bennett E, Weber DJ A quantitative approach to defining "high-touch" surfaces in hospitals. *Infect Control Hosp Epidemiol* 2010; **31**: 850-3.
[446] Aarnisalo K, Lundén J, Korkeala H, Wirtanen G Susceptibility of Listeria monocytogenes strains to disinfectants and chlorinated alkaline cleaners at cold temperatures. *LWT - Food Science and Technology* 2007; **40**: 1041-8.
[447] Berendt AET, L.; Spady, D.; Rennie, R.; Forgie, S. E. Three swipes and you're out: How many swipes are needed to decontaminate plastic with disposable wipes? *Am J Infect Control* 2011; **39**: 442-3.
[448] Best MK, M. E.; Coates, F. Efficacy of a variety of disinfectants against Listeria spp. *Appl Environ Microbiol* 1990; **56**: 377-80.
[449] Calfee MWW, Morgan Inactivation of vegetative bacterial threat agents on environmental surfaces. *Sci Total Environ* 2013; **443**: 387-96.
[450] Calfee MWW, M. Inactivation of Burkholderia pseudomallei on environmental surfaces using spray-applied, common liquid disinfectants. *Lett Appl Microbiol* 2015; **61**: 418-22.
[451] Dellanno CV, Q.; Boesenberg, D. The antiviral action of common household disinfectants and antiseptics against murine hepatitis virus, a potential surrogate for SARS coronavirus. *Am J Infect Control* 2009; **37**: 649-52.
[452] Hulkower RLC, L. M.; Rutala, W. A.; Weber, D. J.; Sobsey, M. D. Inactivation of surrogate coronaviruses on hard surfaces by health care germicides. *Am J Infect Control* 2011; **39**: 401-7.
[453] Jeong EK, Bae JE, Kim IS Inactivation of influenza A virus H1N1 by disinfection process. *Am J Infect Control* 2010; **38**: 354-60.
[454] Kenters N, Huijskens EGW, de Wit SCJ, van Rosmalen J, Voss A Effectiveness of cleaning-disinfection wipes and sprays against multidrug-resistant outbreak strains. *Am J Infect Control* 2017; **45**: e69-e73.
[455] Kim CYR, G. J.; Park, H. Y.; Ryu, K. Resistance of Staphylococcus aureus on food contact surfaces with different surface characteristics to chemical sanitizers. *J Food Saf* 2017; **37**: 9.
[456] Kruse RHG, T. D.; Chambers, R. C.; Jones, M. W. DISINFECTION OF AEROSOLIZED PATHOGENIC FUNGI ON LABORATORY SURFACES. I. TISSUE PHASE. *Appl Microbiol* 1963; **11**: 436-45.
[457] Magulski TP, D.; Bischoff, B.; Becker, B.; Steinmann, E.; Steinmann, J.; Goroncy-Bermes, P. Inactivation of murine norovirus by chemical biocides on stainless steel. *Bmc Infect Dis* 2009; **9**: 7.
[458] Miyano NO, S.; Kamiya, A. Efficacy of disinfectants and hot water against biofilm cells of Burkholderia cepacia. *Biol Pharm Bull* 2003; **26**: 671-4.
[459] Rossvoll EL, S.; Bloomfield, S.; Moen, B.; Heir, E.; Moretro, T. The effects of different hygiene procedures in reducing bacterial contamination in a model domestic kitchen. *J Appl Microbiol* 2015; **119**: 582-93.
[460] Rutala WAP, J. E.; Gergen, M. F.; Sobsey, M. D.; Weber, D. J. Efficacy of hospital germicides against adenovirus 8, a common cause of epidemic keratoconjunctivitis in health care facilities. *Antimicrob Agents Ch* 2006; **50**: 1419-24.
[461] Sattar SA, Bradley C, Kibbee R *et al.* Disinfectant wipes are appropriate to control microbial bioburden from surfaces: use of a new ASTM standard test protocol to demonstrate efficacy. *J Hosp Infect* 2015; **91**: 319-25.
[462] Eterpi MM, G.; Thomas, V. Decontamination efficacy against Mycoplasma. *Lett Appl Microbiol* 2011; **52**: 150-5.

[463] Tiwari S, Rajak S, Mondal DP, Biswas D Sodium hypochlorite is more effective than 70% ethanol against biofilms of clinical isolates of Staphylococcus aureus. *Am J Infect Control* 2018; **46**: E37-E42.
[464] Tyler RA, G. A. J. A surface test for virucidal activity of disinfectants: preliminary study with herpes virus. *J Hosp Infect* 1987; **9**: 22-9.
[465] Udompijitkul PA, Maryam; Sarker, Mahfuzur R. Inactivation strategy for Clostridium perfringens spores adhered to food contact surfaces. *Food Microbiol* 2013; **34**: 328-36.
[466] van Bueren JL, D. P.; Simpson, R. A. Inactivation of human immunodeficiency virus type 1 by alcohols. *J Hosp Infect* 1994; **28**: 137-48.
[467] Whitehead KM, K. A. Virucidal efficacy of disinfectant actives against feline calicivirus, a surrogate for norovirus, in a short contact time. *Am J Infect Control* 2010; **38**: 26-30.
[468] Zeitler BR, I. Surface-Dried Viruses Can Resist Glucoprotamin-Based Disinfection. *Appl Environ Microb* 2014; **80**: 7169-75.
[469] Zonta WM, A.; Farnir, F.; Thiry, E. Comparative Virucidal Efficacy of Seven Disinfectants Against Murine Norovirus and Feline Calicivirus, Surrogates of Human Norovirus. *Food Environ Virol* 2016; **8**: 1-12.
[470] Moretro TV, L. K.; Nesse, L. L.; Storheim, S.; Kotlarz, K.; Langsrud, S. Evaluation of efficacy of disinfectants against Salmonella from the feed industry. *J Appl Microbiol* 2009; **106**: 1005-12.
[471] Bolton SL, Kotwal G, Harrison MA, Law SE, Harrison JA, Cannon JL Sanitizer efficacy against murine norovirus, a surrogate for human norovirus, on stainless steel surfaces when using three application methods. *Appl Environ Microbiol* 2013; **79**: 1368-77.
[472] Herruzo R, Vizcaino MJ, Herruzo I, Sanchez M Surface Disinfectants for Burn Units Evaluated by a New Double Method, Using Microorganisms Recently Isolated From Patients, on a Surface Germ-Carrier Model. *Journal of burn care & research : official publication of the American Burn Association* 2017; **38**: e663-e9.
[473] Malik YS, Allwood PB, Hedberg CW, Goyal SM Disinfection of fabrics and carpets artificially contaminated with calicivirus: relevance in institutional and healthcare centres. *J Hosp Infect* 2006; **63**: 205-10.
[474] Nandy P, Lucas AD, Gonzalez EA, Hitchins VM Efficacy of commercially available wipes for disinfection of pulse oximeter sensors. *Am J Infect Control* 2016; **44**: 304-10.
[475] Lado EAA, Srinath; Stout, Frank W. Efficacy of various surface disinfectants on an irregular surface. *Oral Surgery, Oral Medicine, Oral Pathology* 1993; **75**: 439-42.
[476] Malik YS, Maherchandani S, Goyal SM Comparative efficacy of ethanol and isopropanol against feline calicivirus, a norovirus surrogate. *Am J Infect Control* 2006; **34**: 31-5.
[477] Rutala WAG, M. F.; Weber, D. J. Efficacy of Improved Hydrogen Peroxide against Important Healthcare-Associated Pathogens. *Infect Cont Hosp Ep* 2012; **33**: 1159-61.
[478] McLaren IW, A.; Breslin, M.; Davies, R. Evaluation of commonly-used farm disinfectants in wet and dry models of Salmonella farm contamination. *Avian Pathol* 2011; **40**: 33-42.
[479] Gosling RJM, Ian; Vaughan, Kelly; Davies, Robert H.; Smith, Richard P. Efficacy of disinfectants and detergents intended for a pig farm environment where Salmonella is present. *Vet Microbiol* 2017; **204**: 46-53.
[480] Bahannan SAA-S, M. M. An in-vitro study of the effects of various disinfectants on prosthetic and surface materials. *Saudi Med J* 2002; **23**: 396-9.

[481] Jang YL, J.; So, B.; Lee, K.; Yun, S.; Lee, M.; Choe, N. Evaluation of changes induced by temperature, contact time, and surface in the efficacies of disinfectants against avian influenza virus. *Poult Sci* 2014; **93**: 70-6.

[482] Jang YL, K.; Yun, S.; Lee, M.; Song, J.; Chang, B.; Choe, N. H. Efficacy evaluation of commercial disinfectants by using Salmonella enterica serovar Typhimurium as a test organism. *J Vet Sci* 2017; **18**: 209-16.

[483] Jean JV, J. F.; Moroni, O.; Darveau, A.; Kukavica-Ibrulj, I.; Fliss, I. Effectiveness of commercial disinfectants for inactivating hepatitis A virus on agri-food surfaces. *J Food Prot* 2003; **66**: 115-9.

[484] Juzwa W, Myszka K, Bialas W, Dobrucka R, Konieczny P, Czaczyk K Investigation of the effectiveness of disinfectants against planktonic and biofilm forms of P. aeruginosa and E. faecalis cells using a compilation of cultivation, microscopic and flow cytometric techniques. *Biofouling* 2015; **31**: 587-97.

[485] Malik YS, Goyal SM Virucidal efficacy of sodium bicarbonate on a food contact surface against feline calicivirus, a norovirus surrogate. *Int J Food Microbiol* 2006; **109**: 160-3.

[486] Orsi IAG, A.; Villabona, C. A.; Fernandes, Fhcn; Ito, I. Y. Evaluation of the efficacy of chemical disinfectants for disinfection of heat-polymerised acrylic resin. *Gerodontology* 2011; **28**: 253-7.

[487] Roberts CG, Chan-Myers HB, Favero MS Virucidal activity of ortho-phthalaldehyde solutions against hepatitis B and C viruses. *Am J Infect Control* 2008; **36**: 223-6.

[488] Block CR, E.; Simhon, A.; Shapiro, M. Evaluation of chlorhexidine and povidone iodine activity against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecalis using a surface test. *J Hosp Infect* 2000; **46**: 147-52.

[489] Cabeca TKP, A. C.; Pizzolitto, E. L. Activity of disinfectants against foodborne pathogens in suspension and adhered to stainless steel surfaces. *Braz J Microbiol* 2012; **43**: 1112-9.

[490] Gulati BRA, P. B.; Hedberg, C. W.; Goyal, S. M. Efficacy of commonly used disinfectants for the inactivation of calicivirus on strawberry, lettuce, and a food-contact surface. *J Food Prot* 2001; **64**: 1430-4.

[491] Ban G-HK, Dong-Hyun Effect of sanitizer combined with steam heating on the inactivation of foodborne pathogens in a biofilm on stainless steel. *Food Microbiol* 2016; **55**: 47-54.

[492] Restaino LF, E. W.; Bluestein, R. L.; Hemphill, J. B.; Regutti, R. R. ANTIMICROBIAL EFFICACY OF A NEW ORGANIC-ACID ANIONIC SURFACTANT AGAINST VARIOUS BACTERIAL STRAINS. *J Food Prot* 1994; **57**: 496-501.

[493] Ali SM, M.; Bruce, M.; Jeanes, A.; Moore, G.; Wilson, A. P. R. Efficacy of two hydrogen peroxide vapour aerial decontamination systems for enhanced disinfection of meticillin-resistant Staphylococcus aureus, Klebsiella pneumoniae and Clostridium difficile in single isolation rooms. *J Hosp Infect* 2016; **93**: 70-7.

[494] Barbut FM, D.; Verachten, M.; Girou, E. Comparison of the efficacy of a hydrogen peroxide dry-mist disinfection system and sodium hypochlorite solution for eradication of Clostridium difficile spores. *Infect Control Hosp Epidemiol* 2009; **30**: 507-14.

[495] Barbut FY, Saber; Mimoun, Maurice; Pham, Julien; Chaouat, Marc; Otter, Jonathan A. Reducing the spread of Acinetobacter baumannii and methicillin-resistant Staphylococcus aureus on a burns unit through the intervention of an infection control bundle. *Burns* 2013; **39**: 395-403.

[496] Bentley KD, B. K.; Parks, S. R.; Walker, J. T.; Bennett, A. M. Hydrogen peroxide vapour decontamination of surfaces artificially contaminated with norovirus surrogate feline calicivirus. *J Hosp Infect* 2012; **80**: 116-21.
[497] Berrie EA, L.; Yezli, S.; Otter, J. A. Hydrogen peroxide vapour (HPV) inactivation of adenovirus. *Lett Appl Microbiol* 2011; **52**: 555-8.
[498] Best ELP, P.; Thirkell, G.; Verity, P.; Copland, M.; Else, P.; Denton, M.; Hobson, R. P.; Wilcox, M. H. Effectiveness of deep cleaning followed by hydrogen peroxide decontamination during high Clostridium difficile infection incidence. *J Hosp Infect* 2014; **87**: 25-33.
[499] Blazejewski CW, F.; Rouze, A.; Le Guern, R.; Ponthieux, S.; Salleron, J.; Nseir, S. Efficiency of hydrogen peroxide in improving disinfection of ICU rooms. *Crit Care* 2015; **19**: 8.
[500] Choi NYB, S. Y.; Yoon, J. H.; Choi, M. R.; Kang, D. H.; Lee, S. Y. Efficacy of aerosolized hydrogen peroxide-based sanitizer on the reduction of pathogenic bacteria on a stainless steel surface. *Food Control* 2012; **27**: 57-63.
[501] Goyal SM, Chander Y, Yezli S, Otter JA Evaluating the virucidal efficacy of hydrogen peroxide vapour. *J Hosp Infect* 2014; **86**: 255-9.
[502] Hall LO, J. A.; Chewins, J.; Wengenack, N. L. Deactivation of the dimorphic fungi Histoplasma capsulatum, Blastomyces dermatitidis and Coccidioides immitis using hydrogen peroxide vapor. *Med Mycol* 2008; **46**: 189-91.
[503] Havill NLM, B. A.; Boyce, J. M. Comparison of the Microbiological Efficacy of Hydrogen Peroxide Vapor and Ultraviolet Light Processes for Room Decontamination. *Infect Cont Hosp Ep* 2012; **33**: 507-12.
[504] Herruzo RV, M. J.; Herruzo, I. Quantifying Glosair 400 efficacy for surface disinfection of American Type Culture Collection strains and micro-organisms recently isolated from intensive care unit patients. *J Hosp Infect* 2014; **87**: 175-8.
[505] Holmdahl TW, M.; Uzcategui, N.; Odenholt, I.; Lanbeck, P.; Medstrand, P.; Widell, A. Hydrogen Peroxide Vapor Decontamination in a Patient Room Using Feline Calicivirus and Murine Norovirus as Surrogate Markers for Human Norovirus. *Infect Cont Hosp Ep* 2016; **37**: 561-6.
[506] Johnston MD, Lawson S, Otter JA Evaluation of hydrogen peroxide vapour as a method for the decontamination of surfaces contaminated with Clostridium botulinum spores. *J Microbiol Methods* 2005; **60**: 403-11.
[507] Klapes NAV, D. Vapor-phase hydrogen peroxide as a surface decontaminant and sterilant. *Appl Environ Microbiol* 1990; **56**: 503-6.
[508] Lemmen S, Scheithauer S, Hafner H, Yezli S, Mohr M, Otter JA Evaluation of hydrogen peroxide vapor for the inactivation of nosocomial pathogens on porous and nonporous surfaces. *Am J Infect Control* 2015; **43**: 82-5.
[509] Montazeri N, Manuel C, Moorman E, Khatiwada JR, Williams LL, Jaykus LA Virucidal Activity of Fogged Chlorine Dioxide- and Hydrogen Peroxide-Based Disinfectants against Human Norovirus and Its Surrogate, Feline Calicivirus, on Hard-to-Reach Surfaces. *Front Microbiol* 2017; **8**: 1031.
[510] Otter JAC, M.; Ahmad, F.; van Tonder, C.; Drabu, Y. J. Assessing the biological efficacy and rate of recontamination following hydrogen peroxide vapour decontamination. *J Hosp Infect* 2007; **67**: 182-8.
[511] Steindl GF, A.; Huhulescu, S.; Wewalka, G.; Allerberger, F. Effect of airborne hydrogen peroxide on spores of Clostridium difficile. *Wien Klin Wochen* 2015; **127**: 421-6.
[512] Tuladhar E, Terpstra P, Koopmans M, Duizer E Virucidal efficacy of hydrogen peroxide vapour disinfection. *J Hosp Infect* 2012; **80**: 110-5.
[513] Wood JP, Calfee MW, Clayton M, Griffin-Gatchalian N, Touati A, Egler K Evaluation of peracetic acid fog for the inactivation of Bacillus anthracis

spore surrogates in a large decontamination chamber. *J Hazard Mater* 2013; **250-251**: 61-7.

[514] Wood JPC, M. W.; Clayton, M.; Griffin-Gatchalian, N.; Touati, A.; Ryan, S.; Mickelsen, L.; Smith, L.; Rastogi, V. A simple decontamination approach using hydrogen peroxide vapour for Bacillus anthracis spore inactivation. *J Appl Microbiol* 2016; **121**: 1603-15.

[515] Yui SA, S.; Muzslay, M.; Jeanes, A.; Wilson, A. P. R. Identification of Clostridium difficile Reservoirs in The Patient Environment and Efficacy of Aerial Hydrogen Peroxide Decontamination. *Infect Cont Hosp Ep* 2017; **38**: 1487-92.

[516] Moat JC, J.; Shone, J.; Upton, M. Application of a novel decontamination process using gaseous ozone. *Can J Microbiol* 2009; **55**: 928-33.

[517] Moore GG, C.; Peters, A. Bactericidal properties of ozone and its potential application as a terminal disinfectant. *J Food Prot* 2000; **63**: 1100-6.

[518] Robbins JBF, C. W.; Moltz, A. G.; Martin, S. E. Elimination of Listeria monocytolgenes biofilms by ozone, chlorine, and hydrogen peroxide. *J Food Prot* 2005; **68**: 494-8.

[519] Saha RS, N.; Atwain, A.; Donofrio, R. S. Evaluation of disinfection efficacy of ozone and chlorinated disinfectant against the biofilm of Klebsiella michiganensis and Pseudomonas aeruginosa. *Ann Microbiol* 2014; **64**: 1607-13.

[520] Sharma MH, J. B. Ozone gas is an effective and practical antibacterial agent. *Am J Infect Control* 2008; **36**: 559-63.

[521] de Candia SM, M.; Baruzzi, F. Eradication of high viable loads of Listeria monocytogenes contaminating food-contact surfaces. *Front Microbiol* 2015; **6**: 12.

[522] Mahfoudh AP-É, F.; Moisan, M.; Barbeau, J. Effect of dry-ozone exposure on different polymer surfaces and their resulting biocidal action on sporulated bacteria. *Surface Science* 2010; **604**: 1487-93.

[523] Chander YJ, Thomas; Goyal, Sagar M.; Russell, R. J. Antiviral activity of Ecasol against feline calicivirus, a surrogate of human norovirus. *J Infect Public Heal* 2012; **5**: 420-4.

[524] Deza MAA, M.; Garrido, M. J. Inactivation of Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa and Staphylococcus aureus on stainless steel and glass surfaces by neutral electrolysed water. *Lett Appl Microbiol* 2005; **40**: 341-6.

[525] Li NWL, G. L.; Liu, J. Inactivation of Bacillus cereus biofilms on stainless steel by acidic electrolyzed water. *J Food Process Preserv* 2017; **41**: 6.

[526] Meakin NSB, C.; Lewis, M. R.; Dancer, S. J. Comparison of cleaning efficacy between in-use disinfectant and electrolysed water in an English residential care home. *J Hosp Infect* 2012; **80**: 122-7.

[527] Meireles A, Ferreira C, Melo L, Simoes M Comparative stability and efficacy of selected chlorine-based biocides against Escherichia coli in planktonic and biofilm states. *Food Res Int* 2017; **102**: 511-8.

[528] Moorman EM, N.; Jaykus, L. A. Efficacy of Neutral Electrolyzed Water for Inactivation of Human Norovirus. *Appl Environ Microb* 2017; **83**: 11.

[529] Thorn RM, Robinson GM, Reynolds DM Comparative antimicrobial activities of aerosolized sodium hypochlorite, chlorine dioxide, and electrochemically activated solutions evaluated using a novel standardized assay. *Antimicrob Agents Chemother* 2013; **57**: 2216-25.

[530] Vazquez-Sanchez DC, M. L.; Rodriguez-Herrera, J. J. Single and Sequential Application of Electrolyzed Water with Benzalkonium Chloride or Peracetic Acid for Removal of Staphylococcus Aureus Biofilms. *J Food Saf* 2014; **34**: 199-210.

[531] Wang HC, Linlin; Li, Yunhan; Xu, Xinglian; Zhou, Guanghong Biofilm formation by meat-borne Pseudomonas fluorescens on stainless steel and its resistance to disinfectants. *Food Control* 2018; **91**: 397-403.
[532] Zhao LZ, Y.; Yang, H. S. Efficacy of low concentration neutralised electrolysed water and ultrasound combination for inactivating Escherichia coli ATCC 25922, Pichia pastoris GS115 and Aureobasidium pullulans 2012 on stainless steel coupons. *Food Control* 2017; **73**: 889-99.
[533] Ali SY, S.; Muzslay, M.; Wilson, A. P. R. Comparison of two whole-room ultraviolet irradiation systems for enhanced disinfection of contaminated hospital patient rooms. *J Hosp Infect* 2017; **97**: 180-4.
[534] Armstrong GNW, Ian A.; Stewart-Tull, Duncan E. Inactivation of B. cereus spores on agar, stainless steel or in water with a combination of Nd:YAG laser and UV irradiation. *Innov Food Sci Emerg Technol* 2006; **7**: 94-9.
[535] Bedell KB, A. H.; Perlman, S. Efficacy of an Automated Multiple Emitter Whole-Room Ultraviolet-C Disinfection System Against Coronaviruses MHV and MERS-CoV. *Infect Cont Hosp Ep* 2016; **37**: 598-9.
[536] Bentley JJS, D.; Gram, D. W.; Dujowich, M.; Marsella, R. Can ultraviolet light C decrease the environmental burden of antimicrobial-resistant and -sensitive bacteria on textiles? *Vet Dermatol* 2016; **27**: 457-e121.
[537] Byrns GB, B.; Yang, L.; Webster, K.; Rutherford, G.; Steiner, G.; Petras, D.; Scannell, M. The uses and limitations of a hand-held germicidal ultraviolet wand for surface disinfection. *J Occup Environ Hyg* 2017; **14**: 749-57.
[538] Gabriel AAB, Ma Luisa P.; Rosario, Leo Mendel D.; Tumlos, Roy B.; Ramos, Henry J. Elimination of Salmonella enterica on common stainless steel food contact surfaces using UV-C and atmospheric pressure plasma jet. *Food Control* 2018; **86**: 90-100.
[539] Gostine AG, D.; Donohue, C.; Carlstrom, L. Evaluating the effectiveness of ultraviolet-C lamps for reducing keyboard contamination in the intensive care unit: A longitudinal analysis. *Am J Infect Control* 2016; **44**: 1089-94.
[540] Jean J, Morales-Rayas R, Anoman MN, Lamhoujeb S Inactivation of hepatitis A virus and norovirus surrogate in suspension and on food-contact surfaces using pulsed UV light (pulsed light inactivation of food-borne viruses). *Food Microbiol* 2011; **28**: 568-72.
[541] Kim MP, Shin Young; Ha, Sang-Do Synergistic effect of a combination of ultraviolet–C irradiation and sodium hypochlorite to reduce Listeria monocytogenes biofilms on stainless steel and eggshell surfaces. *Food Control* 2016; **70**: 103-9.
[542] Meyers CM, J.; Robison, R. UVC radiation as an effective disinfectant method to inactivate human papillomaviruses. *Plos One* 2017; **12**: 9.
[543] Moore G, Ali S, Cloutman-Green EA *et al.* Use of UV-C radiation to disinfect non-critical patient care items: a laboratory assessment of the Nanoclave Cabinet. *Bmc Infect Dis* 2012; **12**: 174.
[544] Muzslay M, Yui S, Ali S, Wilson APR Ultraviolet-C decontamination of hand-held tablet devices in the healthcare environment using the Codonics D6000 disinfection system. *J Hosp Infect* 2018; doi 10.1016/j.jhin.2018.04.002.
[545] Nerandzic MMC, J. L.; Pultz, M. J.; Donskey, C. J. Evaluation of an automated ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens in hospital rooms. *Bmc Infect Dis* 2010; **10**: 8.
[546] Nerandzic MMD, C. J. Triggering Germination Represents a Novel Strategy to Enhance Killing of Clostridium difficile Spores. *Plos One* 2010; **5**: 8.
[547] Nerandzic MMT, P.; Sankar, T.; Jencson, A.; Cadnum, J. L.; Ray, A. J.; Salata, R. A.; Watkins, R. R.; Donskey, C. J. Evaluation of a Pulsed Xenon

Ultraviolet Disinfection System for Reduction of Healthcare-Associated Pathogens in Hospital Rooms. *Infect Cont Hosp Ep* 2015; **36**: 192-7.
[548] Park SYK, An-Na; Lee, Ki-Hoon; Ha, Sang-Do Ultraviolet-C efficacy against a norovirus surrogate and hepatitis A virus on a stainless steel surface. *Int J Food Microbiol* 2015; **211**: 73-8.
[549] Friedman NDW, Aaron L.; Boyd, Sarah; Tremonti, Christopher; Low, Jillian; Styles, Kaylene; Harris, Owen; Alfredson, David; Athan, Eugene The effectiveness of a single-stage versus traditional three-staged protocol of hospital disinfection at eradicating vancomycin-resistant Enterococci from frequently touched surfaces. *Am J Infect Control* 2013; **41**: 227-31.
[550] Hendry EC, B.; Worthington, T. Antimicrobial efficacy of a novel eucalyptus oil, chlorhexidine digluconate and isopropyl alcohol biocide formulation. *International journal of molecular sciences* 2012; **13**: 14016-25.
[551] Kawakami HH, T.; Nishikubo, H.; Morikawa, A.; Suzuki, S.; Sato, Y.; Kikuchi, Y. Effects of Surface Contamination and Cleaning with Hypochlorite Wipes on the Antibacterial Activity of Copper-Alloyed Antibacterial Stainless Steel. *Biocontrol Sci* 2014; **19**: 73-8.
[552] Meyer KM, Tufts JA, Calfee MW, Oudejans L Efficacy of sporicidal wipes for inactivation of a Bacillus anthracis surrogate. *J Appl Microbiol* 2014; **117**: 1634-44.
[553] Siani H, Cooper C, Maillard JY Efficacy of "sporicidal" wipes against Clostridium difficile. *Am J Infect Control* 2011; **39**: 212-8.
[554] Siani H, Wesgate R, Maillard JY Impact of antimicrobial wipes compared with hypochlorite solution on environmental surface contamination in a health care setting: A double-crossover study. *Am J Infect Control* 2018; doi 10.1016/j.ajic.2018.03.020.
[555] Verhaelen KB, M.; Rutjes, S.; Husman, A. M. D.; Duizer, E. Wipes Coated with a Singlet-Oxygen-Producing Photosensitizer Are Effective against Human Influenza Virus but Not against Norovirus. *Appl Environ Microb* 2014; **80**: 4391-7.
[556] Williams GJ, Denyer SP, Hosein IK, Hill DW, Maillard JY The development of a new three-step protocol to determine the efficacy of disinfectant wipes on surfaces contaminated with Staphylococcus aureus. *J Hosp Infect* 2007; **67**: 329-35.
[557] Williams GJD, S. P.; Hosein, I. K.; Hill, D. W.; Maillard, J. Y. Limitations of the efficacy of surface disinfection in the healthcare setting. *Infect Control Hosp Epidemiol* 2009; **30**: 570-3.
[558] Diab-Elschahawi MA, Ojan; Blacky, Alexander; Stadler, Maria; Pernicka, Elisabeth; Berger, Jutta; Resch, Helene; Koller, Walter Evaluation of the decontamination efficacy of new and reprocessed microfiber cleaning cloth compared with other commonly used cleaning cloths in the hospital. *Am J Infect Control* 2010; **38**: 289-92.
[559] Frota OPF, Adriano M.; Koch, Rodrigo; de Andrade, Denise; Rigotti, Marcelo A.; Borges, Najla M. A.; Almeida, Margarete T. G. Surface cleaning effectiveness in a walk-in emergency care unit: Influence of a multifaceted intervention. *Am J Infect Control* 2016; **44**: 1572-7.
[560] Gan T, Xu H, Wu JN *et al.* Sequential enhanced cleaning eliminates multidrug-resistant organisms in general intensive care unit of a traditional Chinese medicine hospital. *J Crit Care* 2017; **41**: 216-21.
[561] Gillespie E, Wilson J, Lovegrove A *et al.* Environment cleaning without chemicals in clinical settings. *Am J Infect Control* 2013; **41**: 461-3.
[562] Griffith CJ, Obee P, Cooper RA, Burton NF, Lewis M The effectiveness of existing and modified cleaning regimens in a Welsh hospital. *J Hosp Infect* 2007; **66**: 352-9.

[563] Hamilton DF, A.; Ballantyne, L.; Kingsmore, P.; Bedwell, D.; Hall, T. J.; Hickok, S. S.; Jeanes, A.; Coen, P. G.; Gant, V. A. Performance of ultramicrofibre cleaning technology with or without addition of a novel copper-based biocide. *J Hosp Infect* 2010; **74**: 62-71.
[564] Koo OKM, E. M.; Story, R.; Lindsay, D.; Ricke, S. C.; Crandall, P. G. Comparison of cleaning fabrics for bacterial removal from food-contact surfaces. *Food Control* 2013; **30**: 292-7.
[565] Kusumaningrum HD, Paltinaite R, Koomen AJ, Hazeleger WC, Rombouts FM, Beumer RR Tolerance of Salmonella enteritidis and Staphylococcus aureus to surface cleaning and household bleach. *J Food Prot* 2003; **66**: 2289-95.
[566] Masuku SM, Babu D, Martin EM *et al.* Cleaning and decontamination efficacy of wiping cloths and silver dihydrogen citrate on food contact surfaces. *J Appl Microbiol* 2012; **113**: 89-95.
[567] Meyer KM, Tufts JA, Calfee MW, Oudejans L Efficacy of sporicidal wipes for inactivation of a Bacillus anthracis surrogate. *J Appl Microbiol* 2014; **117**: 1634-44.
[568] Moore G, Griffith C A laboratory evaluation of the decontamination properties of microfibre cloths. *J Hosp Infect* 2006; **64**: 379-85.
[569] Smith DL, Gillanders S, Holah JT, Gush C Assessing the efficacy of different microfibre cloths at removing surface micro-organisms associated with healthcare-associated infections. *J Hosp Infect* 2011; **78**: 182-6.
[570] Trajtman AN, Manickam K, Alfa MJ Microfiber cloths reduce the transfer of Clostridium difficile spores to environmental surfaces compared with cotton cloths. *Am J Infect Control* 2015; **43**: 686-9.
[571] Ali SM, G.; Wilson, A. P. Effect of surface coating and finish upon the cleanability of bed rails and the spread of Staphylococcus aureus. *J Hosp Infect* 2012; **80**: 192-8.
[572] Andersen BM, Rasch M, Kvist J *et al.* Floor cleaning: effect on bacteria and organic materials in hospital rooms. *J Hosp Infect* 2009; **71**: 57-65.
[573] De Lorenzi SR, L.; Finzi, G.; Salvatorelli, G. Biocide activity of microfiber mops with and without silver after contamination. *Braz J Infect Dis* 2011; **15**: 200-3.
[574] Adukwu ECA, S. C.; Phillips, C. A. A comparison of the sensitivity of four Staphylococcus aureus isolates to two chlorine-based disinfectants and an eco-friendly commercially available cleaning agent. *Int J Environ Heal R* 2015; **25**: 115-25.
[575] Almatroudi AG, I. B.; Hu, H.; Jensen, S. O.; Espedido, B. A.; Tahir, S.; Glasbey, T. O.; Legge, P.; Whiteley, G.; Deva, A.; Vickery, K. Staphylococcus aureus dry-surface biofilms are not killed by sodium hypochlorite: implications for infection control. *J Hosp Infect* 2016; **93**: 263-70.
[576] Altieri KTS, P. V.; Machado, A. L.; Giampaolo, E. T.; Pavarina, A. C.; Jorge, J. H.; Vergani, C. E. Eradication of a mature methicillin-resistant Staphylococcus aureus (MRSA) biofilm from acrylic surfaces. *Braz Dent J* 2013; **24**: 487-91.
[577] Andrade NJB, T. A.; Zottola, E. A. Bacteriocidal activity of sanitizers against Enterococcus faecium attached to stainless steel as determined by plate count and impedance methods. *J Food Prot* 1998; **61**: 833-8.
[578] Bagattini MB, R.; Giannouli, M.; Mattiacci, D.; Bellopede, R.; Grimaldi, N.; Nardone, A.; Zarrilli, R.; Triassi, M. Effect of treatment with an overheated dry-saturated steam vapour disinfection system on multidrug and extensively drug-resistant nosocomial pathogens and comparison with sodium hypochlorite activity. *BMC research notes* 2015; **8**: 551.

[579] Bang J, Hong A, Kim H *et al.* Inactivation of Escherichia coli O157:H7 in biofilm on food-contact surfaces by sequential treatments of aqueous chlorine dioxide and drying. *Int J Food Microbiol* 2014; **191**: 129-34.
[580] Byun MWK, J. H.; Kim, D. H.; Kim, H. J.; Jo, C. Effects of irradiation and sodium hypochlorite on the micro-organisms attached to a commercial food container. *Food Microbiol* 2007; **24**: 544-8.
[581] Chatuev BMP, J. W. Analysis of the sporicidal activity of chlorine dioxide disinfectant against Bacillus anthracis (Sterne strain). *J Hosp Infect* 2010; **74**: 178-83.
[582] Chen DZ, T.; Doyle, M. P. Control of pathogens in biofilms on the surface of stainless steel by levulinic acid plus sodium dodecyl sulfate. *Int J Food Microbiol* 2015; **207**: 1-7.
[583] DeQueiroz GAD, D. F. Disinfection of Bacillus subtilis spore-contaminated surface materials with a sodium hypochlorite and a hydrogen peroxide-based sanitizer. *Lett Appl Microbiol* 2008; **46**: 176-80.
[584] DeQueiroz GAD, D. F. Antimicrobial activity and effectiveness of a combination of sodium hypochlorite and hydrogen peroxide in killing and removing Pseudomonas aeruginosa biofilms from surfaces. *J Appl Microbiol* 2007; **103**: 794-802.
[585] Friedrich LMG-S, Renee; Parish, Mickey E.; Danyluk, Michelle D. Mitigation of Alicyclobacillus spp. spores on food contact surfaces with aqueous chlorine dioxide and hypochlorite. *Food Microbiol* 2009; **26**: 936-41.
[586] Hong Y, Teska PJ, Oliver HF Effects of contact time and concentration on bactericidal efficacy of 3 disinfectants on hard nonporous surfaces. *Am J Infect Control* 2017; **45**: 1284-5.
[587] Isakozawa YM, H.; Takesawa, S. Efficacy of Biofilm Removal From Hemodialysis Piping. *Nephro-urology monthly* 2016; **8**: e39332.
[588] Kadurugamuwa JLS, E. Inactivation of human enterovirus 71 and coxsackie virus A16 and hand, foot, and mouth disease. *Am J Infect Control* 2011; **39**: 788-9.
[589] Kean RS, L.; Townsend, E.; McKloud, E.; Short, B.; Akinbobola, A.; Mackay, W. G.; Williams, C.; Jones, B. L.; Ramage, G. Surface disinfection challenges for Candida auris: an in-vitro study. *J Hosp Infect* 2018; **98**: 433-6.
[590] Kim SWB, S. B.; Ha, J. H.; Lee, M. H.; Choi, C.; Ha, S. D. Chlorine Treatment To Inactivate Norovirus on Food Contact Surfaces. *J Food Prot* 2012; **75**: 184-8.
[591] Liu WLL, H. W.; Lee, M. F.; Lin, H. L.; Lin, Y. H.; Chen, C. C.; Chang, P. C.; Lai, C. C.; Chuang, Y. C.; Tang, H. J. The Impact of Inadequate Terminal Disinfection on an Outbreak of Imipenem-Resistant Acinetobacter baumannii in an Intensive Care Unit. *Plos One* 2014; **9**: 7.
[592] Martin HS, C.; Fresnel, R.; Morin, T.; Lamaudiere, S.; Le Sauvage, A. L.; Deleurme, K.; Maris, P. Comparison of the virucidal efficiency of peracetic acid, potassium monopersulfate and sodium hypochlorite on hepatitis A and enteric cytopathogenic bovine orphan virus. *J Appl Microbiol* 2013; **115**: 955-68.
[593] Oie SO, A.; Yamasaki, H.; Furukawa, H.; Kenri, T.; Takahashi, M.; Kawamoto, K.; Makino, S. Disinfection Methods for Spores of Bacillus atrophaeus, B. anthracis, Clostridium tetani, C. botulinum and C. difficile. *Biol Pharm Bull* 2011; **34**: 1325-9.
[594] Park GWS, M. D. Simultaneous comparison of murine norovirus, feline calicivirus, coliphage MS2, and GII.4 norovirus to evaluate the efficacy of sodium hypochlorite against human norovirus on a fecally soiled stainless steel surface. *Foodborne Pathog Dis* 2011; **8**: 1005-10.

[595] Reynolds KAB, S.; Bright, K. R.; Gerba, C. P. Occurrence of Household Mold and Efficacy of Sodium Hypochlorite Disinfectant. *J Occup Environ Hyg* 2012; **9**: 663-9.
[596] Sagripanti JLC, M.; Insalaco, J.; Ziemski, M.; Rogers, J.; Zandomeni, R. Virulent spores of Bacillus anthracis and other Bacillus species deposited on solid surfaces have similar sensitivity to chemical decontaminants. *J Appl Microbiol* 2007; **102**: 11-21.
[597] Sattar SAB, C.; Kibbee, R.; Wesgate, R.; Wilkinson, M. A. C.; Sharpe, T.; Maillard, J. Y. Disinfectant wipes are appropriate to control microbial bioburden from surfaces: use of a new ASTM standard test protocol to demonstrate efficacy. *J Hosp Infect* 2015; **91**: 319-25.
[598] Takahashi KT, R.; Fukuzaki, S. Inactivation of Vibrio parahaemolyticus Unattached and Attached to a Solid Surface in pH-Controlled Sodium Hypochlorite Solutions. *Biocontrol Sci* 2016; **21**: 265-8.
[599] Wagner AG, C. F.; Pedregon, V.; Barth, E.; Gibbs, S. G.; Scarpino, P. V. Inactivation of Bacillus subtilis on gypsum board using aerosolized chemical agents. *J Environ Eng Sci* 2008; **7**: 159-64.
[600] West AMT, P. J.; Lineback, C. B.; Oliver, H. F. Strain, disinfectant, concentration, and contact time quantitatively impact disinfectant efficacy. *Antimicrob Resist In* 2018; **7**: 8.
[601] Yang HK, P. A.; Medeiros, L. C.; Sofos, J. N. Efficacy of sanitizing agents against Listeria monocytogenes biofilms on high-density polyethylene cutting board surfaces. *J Food Prot* 2009; **72**: 990-8.
[602] Yeargin T, Fraser A, Huang GH, Jiang XP Recovery and Disinfection of Two Human Norovirus Surrogates, Feline Calicivirus and Murine Norovirus, from Hard Nonporous and Soft Porous Surfaces. *J Food Prot* 2015; **78**: 1842-50.
[603] Mustapha AL, M. B. DESTRUCTION OF LISTERIA-MONOCYTOGENES BY SODIUM-HYPOCHLORITE AND QUATERNARY AMMONIUM SANITIZERS. *J Food Prot* 1989; **52**: 306-11.
[604] Gallandat K, Lantagne D Selection of a Biosafety Level 1 (BSL-1) surrogate to evaluate surface disinfection efficacy in Ebola outbreaks: Comparison of four bacteriophages. *Plos One* 2017; **12**: e0177943.
[605] Jimenez MM, C. I.; Chaidez, C. Disinfection alternatives for contact surfaces and toys at child care centers. *Int J Environ Heal R* 2010; **20**: 387-94.
[606] Buttner MPC, P.; Stetzenbach, L. D.; Klima-Comba, A. K.; Stevens, V. L.; Cronin, T. D. Determination of the efficacy of two building decontamination strategies by surface sampling with culture and quantitative PCR analysis. *Appl Environ Microbiol* 2004; **70**: 4740-7.
[607] Han Y, Applegate B, Linton RH, Nelson PE Decontamination of Bacillus thuringiensis spores on selected surfaces by chlorine dioxide gas. *J Environ Health* 2003; **66**: 16-21.
[608] Kreske ACR, J. H.; Beuchat, L. R. Evaluation of chlorine, chlorine dioxide, and a peroxyacetic acid-based sanitizer for effectiveness in killing Bacillus cereus and Bacillus thuringiensis spores in suspensions, on the surface of stainless steel, and on apples. *J Food Prot* 2006; **69**: 1892-903.
[609] Li YJ, Zhu N, Jia HQ, Wu JH, Yi Y, Qi JC Decontamination of Bacillus subtilis var. niger spores on selected surfaces by chlorine dioxide gas. *Journal of Zhejiang University Science B* 2012; **13**: 254-60.
[610] Morino H, Fukuda T, Miura T, Shibata T Effect of low-concentration chlorine dioxide gas against bacteria and viruses on a glass surface in wet environments. *Lett Appl Microbiol* 2011; **53**: 628-34.
[611] Nam H, Seo HS, Bang J, Kim H, Beuchat LR, Ryu JH Efficacy of gaseous chlorine dioxide in inactivating Bacillus cereus spores attached to and in a biofilm on stainless steel. *Int J Food Microbiol* 2014; **188**: 122-7.

[612] Park SHK, D. H. Influence of surface properties of produce and food contact surfaces on the efficacy of chlorine dioxide gas for the inactivation of foodborne pathogens. *Food Control* 2017; **81**: 88-95.
[613] Park SH, Kang DH Effect of temperature on chlorine dioxide inactivation of Escherichia coli O157:H7, Salmonella typhimurium, and Listeria monocytogenes on spinach, tomatoes, stainless steel, and glass surfaces. *Int J Food Microbiol* 2018; **275**: 39-45.
[614] Rastogi VKR, S. P.; Wallace, L.; Smith, L. S.; Shah, S. S.; Martin, G. B. Systematic Evaluation of the Efficacy of Chlorine Dioxide in Decontamination of Building Interior Surfaces Contaminated with Anthrax Spores. *Appl Environ Microb* 2010; **76**: 3343-51.
[615] Ryu JHB, L. R. Biofilm formation and sporulation by Bacillus cereus on a stainless steel surface and subsequent resistance of vegetative cells and spores to chlorine, chlorine dioxide, and a peroxyacetic acid-based sanitizer. *J Food Prot* 2005; **68**: 2614-22.
[616] Sabbah S, Springthorpe S, Sattar SA Use of a mixture of surrogates for infectious bioagents in a standard approach to assessing disinfection of environmental surfaces. *Appl Environ Microbiol* 2010; **76**: 6020-2.
[617] Shirasaki YM, A.; Uekusa, M.; Ito, Y.; Hayashi, T. A study of the properties of chlorine dioxide gas as a fumigant. *Exp Anim* 2016; **65**: 303-10.
[618] Foschino R, Nervegna I, Motta A, Galli A Bactericidal activity of chlorine dioxide against Escherichia coli in water and on hard surfaces. *J Food Prot* 1998; **61**: 668-72.
[619] Block C The effect of Perasafe((R)) and sodium dichloroisocyanurate (NaDCC) against spores of Clostridium difficile and Bacillus atrophaeus on stainless steel and polyvinyl chloride surfaces. *J Hosp Infect* 2004; **57**: 144-8.
[620] Morgenthau AN, A. M.; Laursen, A. E.; Foucher, D. A.; Wolfaardt, G. M.; Hausner, M. Assessment of the working range and effect of sodium dichloroisocyanurate on Pseudomonas aeruginosa biofilms and planktonic cells. *Biofouling* 2012; **28**: 111-20.
[621] Williams GJD, S. P.; Hosein, I. K.; Hill, D. W.; Maillard, J. Y. Use of sodium dichloroisocyanurate for floor disinfection. *J Hosp Infect* 2009; **72**: 279-81.
[622] Bae YM, Baek SY, Lee SY Resistance of pathogenic bacteria on the surface of stainless steel depending on attachment form and efficacy of chemical sanitizers. *Int J Food Microbiol* 2012; **153**: 465-73.
[623] Chaitiemwong NH, W. C.; Beumer, R. R. Inactivation of Listeria monocytogenes by Disinfectants and Bacteriophages in Suspension and Stainless Steel Carrier Tests. *J Food Prot* 2014; **77**: 2012-20.
[624] Conlon-Bingham GA, M.; Kearney, M. P.; Scott, M. G.; Baldwin, N.; McElnay, J. C. Reduction in the incidence of hospital-acquired MRSA following the introduction of a chlorine dioxide 275ppm based disinfecting agent in a district general hospital. *Eur J Hosp Pharm-Sci Pract* 2016; **23**: 28-32.
[625] Lee JS, Bae YM, Lee SY, Lee SY Biofilm Formation of Staphylococcus aureus on Various Surfaces and Their Resistance to Chlorine Sanitizer. *J Food Sci* 2015; **80**: M2279-86.
[626] Marques SCR, Jdos; Alves, L. A. D.; Silva, B. C.; Alves, E.; de Abreu, L. R.; Piccoli, R. H. Formation of biofilms by Staphylococcus aureus on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. *Braz J Microbiol* 2007; **38**: 538-43.
[627] Murray JM, T.; Gill, C. I. R.; Kearney, M. P.; Farren, D.; Scott, M. G.; McMullan, G.; Ternan, N. G. Evaluation of bactericidal and anti-biofilm properties of a novel surface-active organosilane biocide against healthcare

associated pathogens and Pseudomonas aeruginosa biolfilm. *Plos One* 2017; **12**: 13.
[628] Vohra PP, I. R. Efficacy of decontaminants and disinfectants against Clostridium difficile. *J Med Microbiol* 2011; **60**: 1218-24.
[629] Sangsriratanakul NT, Chiharu; Suzuki, Mayuko; Komura, Miyuki; Yamada, Masashi; Alam, Md Shahin; Ruenphet, Sakchai; Shoham, Dany; Sakai, Kouji; Takehara, Kazuaki Virucidal efficacy of food additive grade calcium hydroxide against surrogate of human norovirus. *J Virol Methods* 2018; **251**: 83-7.
[630] Julian TRT, J. M.; Schwab, K. J. Evaluating Efficacy of Field-Generated Electrochemical Oxidants on Disinfection of Fomites Using Bacteriophage MS2 and Mouse Norovirus MNV-1 as Pathogenic Virus Surrogates. *Food Environ Virol* 2014; **6**: 145-55.
[631] World Health Organization. How to handrub: How to handwash. In: Care CCiS, Ed.: World Health Organization 2006.

APPENDIX

Supplementary information for chapter 2

Table S.1 Search terms used for surface sampling literature review

Search terms
Hospital AND surface
Hospital AND environmental monitoring
Hospital AND surface sampling
Hospital AND surface AND swab
Hospital AND surface AND contact plate
Hospital AND surface AND sponge
Hospital AND contamination NOT outbreak
Hospital AND environment
Hospital AND surface AND organisms
Hospital AND surface AND infection
Hospital AND surface NOT outbreak
Hospital AND surface NOT outbreak NOT review
Dipslide AND efficacy
Dipslide AND surface
Contact plate AND efficacy
Contact plate AND surface
Contact plate AND environmental monitoring
Sponge AND efficacy
Sponge AND surface
Sponge AND environmental monitoring
Swab AND efficacy
Swab AND surface
Swab AND environmental monitoring
Petrifilm AND efficacy

Petrifilm AND surface
Petrifilm AND environmental monitoring
Surface AND sampling
Surface AND sampling AND efficacy
Environmental monitoring AND surface
Environmental AND monitoring
Environment monitoring AND surface

Supplementary information for chapter 3

Table S2. Results for *S. aureus* recovered with all surface sampling devices across all surface materials.

S. aureus						
	Ceramic		Plastic		Steel	
	Recovery (%) Range (%)	Standard Deviation	Recovery (%) Range (%)	Standard Deviation	Recovery (%) Range (%)	Standard Deviation
Dipslide	38.79	16.65	56.44	11.95	56.27	10.95
	9.09-63.64		36.05-77.90		40.56-65.02	
Petrifilm	23.72	5.57	31.82	9.40	29.43	10.23
	16.39-36.07		22.64-50.94		7.55-45.28	
Cotton swab	2.64	0.35	0.43	0.74	1.01	1.04
	2.00-3.31		0-2.02		0-3.54	
Flocked swab	5.44	0.28	1.16	0.64	1.35	0.78
	4.65-5.69		0-2.03		0.68-2.03	
Sponge	0.63	0.18	0.02	0.03	0.01	0.03

	0.44-1.00		0-0.07		0-0.07	
Contact plate	39.29	4.70	55.81	73.10	47.47	9.89
	32.16-47.45		25.75-83.35		28.31-67.43	

Table S3. Results for *K. pneumoniae* recovered with all surface sampling devices across all surface materials.

K. pneumoniae						
	Ceramic		Plastic		Steel	
	Recovery (%) Range (%)	Standard Deviation	Recovery (%) Range (%)	Standard Deviation	Recovery (%) Range (%)	Standard Deviation
Dipslide	63.58	1.35	50.28	19.86	37.55	5.93
	60.94-66.09		20.48-68.60		29.73-41.93	
Petrifilm	34.91	1.38	20.95	5.77	22.77	8.20
	32.62-38.63		11.69-29.87		14.29-38.31	
Cotton swab	2.69	0.09	0.59	0.69	1.28	0.70
	2.60-2.89		0-2.70		0-2.36	
Flocked swab	6.08	0.15	0.89	0.44	1.30	0.86
	5.90-6.19		0-1.22		0-2.74	
Sponge	0.95	0.06	0.03	0.07	0.01	0.05
	0.88-1.12		0-0.20		0-0.20	
Contact plate	58.26	4.01	44.31	19.15	30.93	9.34
	52.17-63.98		22.88-96.61		20.34-50.85	

Table S4. Results for *P. aeruginosa* recovered with all surface sampling devices across all surface materials.

P. aeruginosa						
	Ceramic		Plastic		Steel	
	Recovery (%) Range (%)	Standard Deviation	Recovery (%) Range (%)	Standard Deviation	Recovery (%) Range (%)	Standard Deviation
Dipslide	65.59	4.66	12.16	4.06	14.87	1.45
	60.87-79.13		3.85-18.37		12.96-17.41	
Petrifilm	26.52	1.95	6.87	6.04	21.35	15.13
	22.61-30.43		1.30-22.08		3.52-49.90	
Cotton swab	1.10	0.09	1.29	2.09	1.25	4.07
	0.99-1.30		0-2.94		0-15.85	
Flocked swab	1.60	0.08	0.32	0.53	2.40	2.76
	1.49-1.84		0-1.66		0-10.60	
Sponge	0.13	0.13	0.03	0.06	0.06	0.09
	0.03-5.53		0-0.14		0-0.28	
Contact plate	33.59	6.50	2.84	1.03	11.86	5.51
	23.53-43.92		1.50-5.26		2.76-23.05	

Table S5. Results for *E. faecalis* recovered with all surface sampling devices across all surface materials.

E. faecalis						
	Ceramic		Plastic		Steel	
	Recovery (%) Range (%)	Standard Deviation	Recovery (%) Range (%)	Standard Deviation	Recovery (%) Range (%)	Standard Deviation
Dipslide	69.46	8.26	43.57	18.84	64.68	10.68
	58.06-83.87		19.69-74.02		48.03-80.31	
Petrifilm	35.05	6.20	16.83	6.40	41.83	17.35
	22.58-45.16		6.09-25.51		14.35-78.41	
Cotton swab	3.78	0.72	4.50	6.02	10.08	8.36
	3.27-4.88		0.23-21.61		1.64-22.79	
Flocked swab	5.84	0.50	0.67	0.53	1.18	1.05
	4.83-6.58		0-1.86		0-4.46	
Sponge	0.80	0.46	0.04	0.08	0.06	0.11
	0.25-1.38		0-0.19		0-0.37	
Contact plate	51.75	8.98	3.36	3.35	54.97	9.40
	38.10-71.43		0-13.75		35.47-68.36	

Supplementary content for chapter 4

Table S6-S13 Breakdown of all literature and cleaning agents recovered from review

Alcohols				
Ethanol	*Isopropanol*	*Methanol*	*Ethyl alcohol*	*Benzyl alcohol*
[446] [351] [447] [448] [449] [450] [451] [452] [453] [454] [455] [456] [457] [458] [459] [460] [461] [462] [463] [464] [465] [466] [467] [468] [469] [470]	[446] [351] [447] [471] [449] [472] [453] [457] [473] [474] [460] [461] [466] [467] [469]	[464] [466]	[451] [475] [476]	[477]

Aldehydes			
Formaldehyde	*Glutaraldehyde*	*Ortho-phthalaldehyde*	*Dialdehyde*
[478] [352] [479] [456]	[478] [477] [464] [469] [480] [352] [479] [481] [482] [483] [484] [457] [473] [485] [486] [475] [470]	[487] [477] [452]	[464] [485]

Iodine-based	
iodine	*iodophor*
[480] [352] [488] [489] [479] [490]	[491] [352] [456] [492] [464] [465]

[456] [478] [458] [460]

Terpenes
[449] [450] [451] [467]

Oxidising agents			
Hydrogen peroxide vapour	***ozone***	***Potassium persulfate***	***Electrolysed water***
[493] [494] [495] [496] [497] [498] [499] [343] [500] [501] [502] [503] [504] [505] [506] [507] [508] [509] [510] [511] [512] [513] [514] [515] [469]	[516] [517] [518] [519] [520] [521] [522]	[351] [470]	[523] [524] [525] [526] [527] [528] [529] [530] [531] [532]

UV and UV-C
[533] [534] [535] [536] [537] [538] [539] [503] [540] [541] [542] [543] [544] [545] [546] [547] [548]

Physical cleaning methods		
Wipes	***Cloths***	***Mops***
[533] [447] [471] [549] [374] [550] [551] [454] [552] [474] [345] [459] [461] [553] [554] [555] [556] [557]	[558] [549] [559] [560] [561] [562] [563] [472] [564] [565] [566] [567] [568] [459] [554] [569] [570] [571] [406]	[572] [573] [4] [549] [346]

Chlorine							
Sodium hypochlorite	*Calcium hypochlorite*	*Chloramine*	*Chlorine dioxide*	*Sodium hydroxide*	*Sodium dichlorocynaurate*	*Calcium hydroxide*	*Electrochemical oxidants*
[351] [574] [575] [576] [577] [578] [480] [491] [579] [494] [448] [580] [489] [581] [582] [353] [451] [583] [584] [524] [585] [374] [354] [490] [472] [586] [452] [587] [483] [588] [551] [589] [455] [541] [590] [456] [591] [592] [458] [474] [593] [486] [594] [595] [459] [460] [596] [519] [597] [598] [529] [463] [464] [349] [599] [531] [600] [467] [601] [602]	[604]	[448] [549]	[585] [579] [606] [581] [607] [472] [483] [608] [609] [527] [509] [610] [611] [612] [613] [614] [615] [616] [617] [529] [599] [531] [618]	[462] [470]	[351] [574] [619] [604] [527] [620] [464] [348] [621] [622] [448] [623] [624] [549] [481] [482] [625] [626] [627] [628]	[629]	[630]

[469] [527] [552] [603]

[604] [605] [470]

Supplementary content for chapter 8

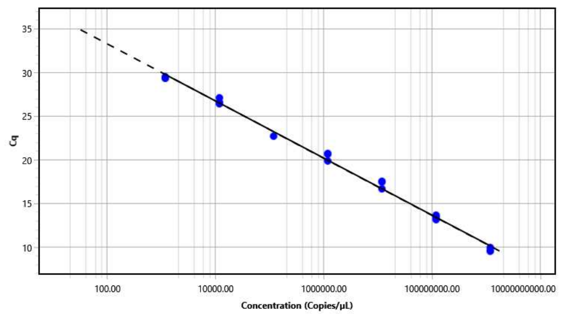
Table S14 Temperature and humidity recordings taken during viability over time testing of DNA marker

Time of sampling (hrs)	0	2	4	6	8	24	48	72	7 days	14 days	30 days
Temperature (^oC)	21.1	21	21.1	21.1	21.7	21.3	20.3	20.3	21.1	21.1	21
Humidity (%)	17	17	17	17	17	18	17	17	17	17	17

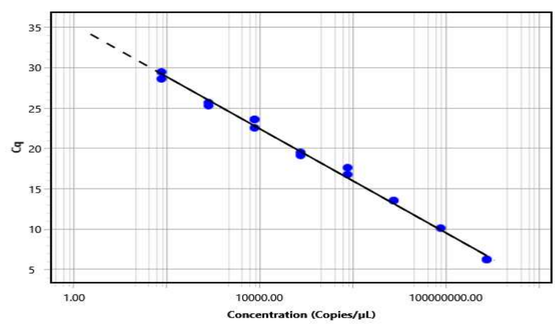
Table S15 Temperature and humidity recordings taken during DNA marker transfer and hand hygiene assessments

Experiment	Temperature (^oC)	Humidity (%)
DNA transfer, gloved hands	21.6	17
DNA transfer, ungloved (bare) hands	21.1	17
Hand hygiene	22	17
Wipe removal test	21.6	17

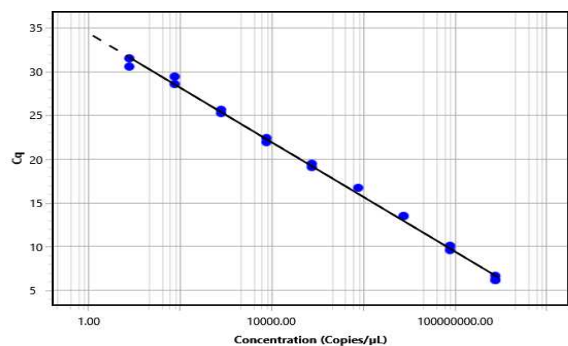
Cauliflower 1: R2 0.99 efficiency: 102%



Cauliflower 2: R2 0.99 106



Cauliflower 3: R2 0.99 efficiency 108%



Figures S1-S3 qPCR assay efficiencies

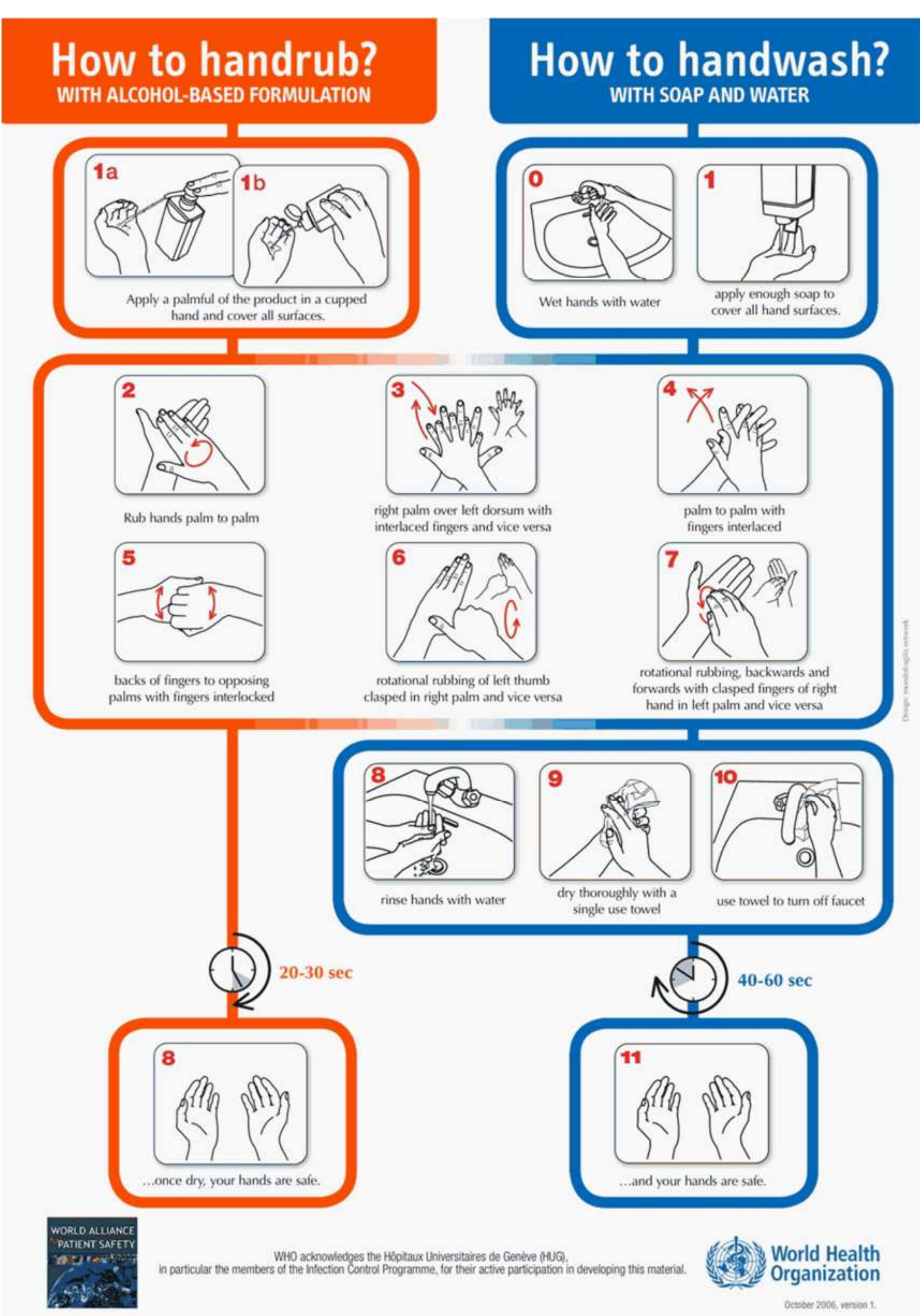


Figure S4. How to hand wash – guidelines followed for hand hygiene removal of DNA testing as taken from As taken from [631]